University of Alberta

Regional Bioremediation Facilities Process Optimization and Monitoring

by

Marie-Christine Bouchard

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Environmental Engineering

Department of Civil Engineering

Edmonton, Alberta

Fall 1996

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ISBN 0-612-18239-8

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Degree: Master of Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Regional Bioremediation Facilities Process Optimization and Monitoring submitted by Marie-Christine Bouchard in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering.

Dr. S.J. Stanley

Dr. D. Smith

Dr. P. Fedorak

To my husband, Capt. Michael Robert Annis, for his unconditional love and support.

Abstract

Interprovincial Pipe Line Inc. (IPL) operates Regional Bioremediation Facilities (RBF) for the treatment of petroleum-contaminated soils which they recover from their operations. The overall objective of this study was to better understand operating conditions and practices necessary to optimize performance of IPL's RBFs. Soil monitoring was conducted at the Edmonton RBF throughout the summer of 1995 and the results are discussed. It was found that with frequent tilling and nutrient addition, remediation objectives were met. Based on the monitoring results, a bench-scale optimization study was conducted to further analyze the effects of aggregate size reduction and inorganic nutrient addition on the bioremediation of petroleum contaminated soils. This study indicated that addition of inorganic nutrient had a significant positive effect on the extent of hydrocarbon removal within an eight-week period but that there was no advantage to further processing of the soil to reduce aggregate size from 5 to 7 cm in diameter to 0.5 cm in diameter.

Acknowledgements

I would first like to thank Dr. Steve Stanley, whom I greatly appreciated working with, for always being available during this project and for his common-sense approach in directing my research.

I would like to sincerely thank Sandra Kenefick without whose help I would not have survived in the lab, Greg Milne from IPL who made this project possible and Faizal Hasham whose invaluable help kept this project on schedule.

I would also like to acknowledge the following people for their contribution to this project: Karla Schumacker, Nick Chernucka, Sheldon Lovell and all the gentlemen at the PLM Shop at IPL.

I also acknowledge the financial support from Interprovincial Pipe Line Inc. and Province of Alberta Graduate Scholarship.

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List of Abbreviations

A	Area
AS	Aggregate size reduction
CA	Contaminant added
CCME	Canadian Council of Ministers of the Environment
CFU	Colony-forming units
COV	Coefficient of variation
CS	Contaminated soil
DO	Diesel oil
DRO	Diesel range organics
EC	Electrical conductivity
E.T.	Enviro-Test Laboratories
GC	Gas chromatography
HUB	Hydrocarbon utilizing bacteria
ICP	Inductively coupled plasma
IPL	Interprovincial Pipe Line Inc.
LPL	Lakehead Pipe Line Company
MDL	Method detection limit
NA	Nutrient adjustment
NEB	National Energy Board
OC	Oil concentration
PAH	Polynuclear aromatic hydrocarbons
RBF	Regional Bioremediation Facility
SAR	Sodium Adsorption Ratio
T.E.H.	Total extractable hydrocarbons
THB	Total heterotrophic bacteria
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
TRPH	Total recoverable petroleum hydrocarbons
WSF	Water-soluble fraction

1.0 Introduction

1.1 Thesis Organization

This thesis is organized in five sections. This first section is the introduction to the thesis in which some background information on the research project and the study objectives are presented. The second section includes the literature review where specific information about the research topic is presented. In the third section all information pertaining to the field work component of this research is presented. The fourth section pertains to the bench-scale optimization experiment conducted in this research project. Finally, in the fifth section conclusions from this research project are presented and recommendations for further studies are made.

1.2 Background

1.2.1 Interprovincial Pipe Line Inc. Regional Bioremediation Facility - Edmonton

Interprovincial Pipe Line Inc. (IPL) operates four Regional Bioremediation Facilities (RBF) in western Canada for the treatment of petroleum-contaminated soils which they recover from their operations. The Edmonton RBF is located in East Edmonton, southeast of the junction of Baseline Road and 17 Street. It consists of four soil treatment cells and a runoff collection pond. Seven groundwater monitoring wells are dispersed around the treatment area and there are two surface water bodies north and south of the facility. The facility layout is presented at Appendix A.

The Edmonton RBF has been in operation since 1994 and soils have been actively treated in Cells A, C and D while Cell B has been used to stockpile soils awaiting treatment. The facility is operated between May and October. The basic operational procedure consists of (1) loading the soils in the treatment cells, (2) performing detailed organic and inorganic analyses on the soils to assess suitability of soil conditions for bioremediation, (3) treating the soils and (4) performing routine organic and inorganic analyses aimed at monitoring decreases in hydrocarbon levels.

Treatment at the facility includes tilling, adjusting the nutrient contents to a target molar C:N:P ratio of 100:5:1 and adding water to adjust the soil moisture content as required.

The operation of the RBFs is regulated by the National Energy Board (NEB). The remediation target set by the agency is 2000 mg/kg hydrocarbon concentration. In practice, Total Extractable Hydrocarbons (C_7 - C_{30}) (T.E.H. (C_7 - C_{30})) concentrations are monitored and the soils in a cell are considered to be remediated when the 90% confidence limit on the mean of six samples is equal to or less than the numerical remediation target. Other soil parameters are also assessed against Canadian Council of Ministers of the Environment (CCME) interim criteria for remediation guidelines (CCME, 1991) in order to identify any potential problems associated with reusing the remediated soils in industrial applications.

In accordance with the NEB orders, groundwater and surface water samples are analyzed throughout the operating season to evaluate effects on groundwater quality and/or identify other environmental issues. When applicable, results are assessed against CCME interim criteria for water quality guidelines (CCME, 1991). Waterfowl and other wildlife are also monitored at the facility to identify any concerns.

1.2.2 1994 Monitoring Results

In 1994 the monitoring of the Edmonton RBF was performed by CH2M Hill Engineering Ltd. This section highlights some of their findings.

Two of the cells had initial concentrations of (T.E.H.) (C_7 - C_{60}) of approximately 8000 mg/kg, while the third had a starting T.E.H. (C_7 - C_{60}) concentration of approximately 6000 mg/kg. Significant reductions in T.E.H. were not observed in any cell over the 1994 season, although a loss of lighter hydrocarbons and reduction in soil toxicity was experienced.

Wet soil conditions prevailed throughout June and July 1994 due to weather conditions and drainage problems within the cells. As a result the soils could not be tilled between late May and late August. Microbial analysis indicated that the soils supported a viable population of hydrocarbon-utilizing bacteria, soil pH conditions were near-neutral and toxic or inhibitory substances were not identified at concentrations likely to impede microbial growth. Although nitrogen concentrations were below

optimal levels they were judged to be such that they would not prevent bioremediation, nor explain the apparent lack of any degradation that year as the soils at a different RBF experienced a considerable reduction in T.E.H. with similar C:N ratios. It was speculated at that time that the very wet soil conditions and fine soil texture (silty clay) may have combined to limit oxygen diffusion and, hence, biodegradation (CH2M Hill Engineering Ltd., 1994). It was also reported that the operation of the facility had no discernible effect on groundwater quality and environmental issues were not identified.

1.3 Study Objectives

The overall objective of this study was to better understand operating conditions and practices necessary to optimize performance of IPL's RBFs. In order to accomplish this objective, specific research tasks were developed. The first specific task was to conduct soil monitoring activities at the Edmonton RBF. Based on these soil monitoring results, two factors were identified for further investigation of their effects with respect to optimizing the bioremediation process.

The second task was then to perform a bench-scale optimization study to analyze the effects of aggregate size reduction and inorganic nutrient addition on the bioremediation of petroleum contaminated soils. Finally, field monitoring and bench-scale study results were analyzed to identify important treatment factors and confirm important design parameters.

2.0 Literature Review

2.1 Literature Review Organization

Presented is a brief review of the important aspects of ex-situ surface bioremediation of petroleum hydrocarbon-contaminated soils. First, a definition is offered followed by a review of the biodegradation process characteristics. Secondly, the soil environment is described with respect to its physical, chemical and microbiological properties; the effects of different soil conditions on the bioremediation process are also reviewed. Thirdly, characteristics of petroleum hydrocarbons as contaminants are reviewed on the basis of their physico-chemical properties, bioavailability, biodegradability and susceptibility to abiotic transformation processes. Finally, petroleum contaminated soil cleanup criteria and ex-situ bioremediation process applications are reviewed.

2.2 Process Characterization

2.2.1 Definition: Ex-Situ Surface Bioremediation

Surface bioremediation utilizes natural soil microorganisms to degrade organic contaminants into cell matter, carbon dioxide, water and inorganic and organic end products. In its simplest form, ex-situ surface bioremediation involves spreading the contaminated soil on an impermeable surface and tilling it to promote the aerobic degradation of the contaminants; water and dissolved nutrients may be added to the soil to promote degradation.

2.2.2 Biodegradation Process

2.2.2.1 General

In this section, information on the microorganisms and the physical and biochemical pathways involved in the bioremediation process is presented. Next, the different kinetic models used to describe the process are reviewed. Potential products of

the bioremediation process are also described. Finally, the effects of bioremediation on contaminant toxicity are reviewed.

2.2.2.2 Microorganisms

Microorganisms can break down hydrocarbons via fermentation, aerobic respiration and anaerobic respiration processes. However, as aerobic biodegradation occurs via more efficient and rapid metabolic pathways and end products of anaerobic degradation include reduced compounds some of which are toxic to microorganisms and plants, soil decontamination is usually conducted under aerobic conditions (Riser-Roberts, 1992, Frankenberger, 1992, King et al., 1992).

Biodegradation of crude and refined oils occurs by the cooperative effort of a mixed microbial community of bacteria, filamentous fungi, and yeasts (Bossert and Bartha, 1984, Riser-Roberts, 1992, King et al., 1992). Bacteria tend to respond more rapidly to oil contamination of soil, whereas fungi may be inhibited initially; conversely, the activity of fungi tends to persist long after bacterial activity has tapered off (Bossert and Bartha, 1984). It has also been shown that filamentous fungi and yeast biodegradation is more limited in its ability to act on a wide range of hydrocarbons, showing a preference for *n*-alkanes over aromatic hydrocarbons and are considered to have a significant degradative role only under stressed conditions such as low pH, for example (Foght and Westlake, 1987). Protozoa may act as vectors to introduce some petroleum components into the food chain by grazing on hydrocarbonoclastic bacteria and yeasts (Bossert and Bartha, 1984).

The heterotrophic bacteria are amongst the most important organisms in the transformation of organic compounds, and soil treatment schemes may be directed toward enhancing their activity (Riser-Roberts, 1992). Heterotrophs can use the organic contaminants as sources of both carbon and energy. It is thought that all soils, except those that are very acidic, contain organisms capable of degrading oil products; no place has been found in the United States or Canada at depths to 122 m where sufficient organisms were not present to allow a significant population within 72 hours (Riser-Roberts, 1992). The actual types and abundance of microorganisms depend upon the local climate, vegetation, soil and the types of contaminants to which the organisms have

been exposed (Fan and Tafuri, 1994). The bacterial genera that are most often isolated from oil-contaminated soils are *Pseudomonas, Arthrobacter, Alcaligenes, Corynbacterium, Flavobacterium, Achromobacter, Micrococcus, Nocardia,* and *Mycobacterium* (Wang and Bartha, 1994). The soil fungi most often isolated as hydrocarbon utilizers are in decreasing order *Trichoderma, Penicillium, Aspergillus and Mortierella* (Bossert and Bartha, 1984).

As mentioned above, the types of hydrocarbons and their concentration will influence the characteristics of the microbial population; it is also reported that different types of petroleum products support different populations of microorganisms (Walker et al., 1976). Studies have also shown that the microbial community changes during remediation (Wang and Bartha, 1994, Compeau et al., 1991, Brown et al., 1983) Specifically, the species diversity is altered and is enriched in hydrocarbon-utilizing microorganisms while, in general, other heterotrophic organisms, that utilize various hydrocarbon biodegradation intermediates, decrease in proportion but may increase in numbers. It has been observed that hydrocarbon-utilizing bacteria (HUB) can represent as high as 50 to 80% of the total bacteria (Oudot et al., 1987). This enrichment in HUB was found to be significantly greater when bioremediation measures such as pH control, mineral nutrients and aeration (tilling) were used (Wang and Bartha, 1994). In a study on microbial degradation of fuel oil in soil microcosms a 1000-fold increase in HUB and a 100-fold increase in total heterotrophic bacteria (THB) were observed after 16 days in contaminated soils; up to 56% of the bacterial population was adapted to hydrocarbon assimilation (Chaîneau et al., 1995). In some cases it has been observed that the microbial numbers first declined after hydrocarbon exposure of the soil, followed by a subsequent increase in microbial numbers. As the biodegradable contaminants are reduced, the microbial community rapidly returns to its pre-contamination steady-state (Wang and Bartha, 1994).

2.2.2.3 Physical Pathways

Microbes are limited to soluble materials that can be transported across their cell membranes into the interior cellular fluid where digestion takes place (although digestion can take place outside bacterial cells in certain instances) (King et al., 1992).

If the organic is especially insoluble, then the colony will secrete extracellular enzymes and surfactants that solubilize the pollutant for use as food inside the cell (King et al., 1992). Other pathways have been suggested and will be discussed later.

Most microorganisms in the subsurface are firmly attached to soil particles and are restricted to the moist environment because they require water to live. Microbial movement to the substrate is unlikely; it has been suggested that active bacterial movement will only be important in microenvironments and probably does not contribute greatly to their widespread distribution in soil (McGill, 1978). Nutrients must therefore be brought to the microbes by advection or diffusion through the mobile phases i.e. water and soil gas. In the simplest and perhaps most common case, the compound to be consumed for energy and cell synthesis is brought into aqueous solution by infiltrating water. At the same time, oxygen, the electron acceptor used to oxidize the carbon source is brought by diffusion through the soil gas. In unsaturated soil, volatile hydrocarbons can also move readily as vapors in the soil gas.

2.2.2.4 Biochemical Pathways

Because of the complexity of the substrate, petroleum hydrocarbon biodegradation should be considered as a synergistic process involving different species with varying degradative capabilities (Foght and Westlake, 1987, Fan and Tafuri, 1994). Very generally, the reaction can be depicted as:

$$\text{Substrate} + O_2 \Rightarrow \text{Biomass} + CO_2 + H_2O + \text{Other Organics} \qquad (1)$$

The general degradation pathway for an alkane involves terminal oxidation to sequentially form a primary alcohol (first stable intermediate), an aldehyde and a fatty acid. For a straight-chain alkane, often only the monocarboxylic acid is observed and branched alkanes degrade to dicarboxylic acid (Fan and Tafuri, 1994). The diterminal oxidation required for branched alkanes degradation make them less readily biodegradable (Bartha, 1986). The fatty acid is then sequentially cleaved, releasing an acetyl coenzyme A that is eventually converted to carbon dioxide and forming a new fatty acid two carbon units shorter than the parent molecule; this process is known as beta oxidation (Riser-Roberts, 1992). An alternative pathway is through subterminal oxidation which involves the sequential formation of a secondary alcohol and a ketone

and the final production of acetate and a long-chain alcohol that is degraded further through beta oxidation (Schneider and Billingsley, 1990). In both cases the initial enzymatic attack involves a class of enzymes called oxygenases.

Cycloalkanes are transformed by a not fully characterized (Bartha, 1986) oxidase system to a corresponding cyclic alcohol which is dehydrogenated to ketone. In the next step, a monooxygenase system distinctly different from the previously mentioned oxidase lactonizes the ring, which is subsequently opened by a lactone hydrolase.

The initial activation and oxidation of aromatic hydrocarbons involve enzymes called oxygenases produced by the microorganisms that catalyze oxygen-fixing reactions. Fungi generally produce monooxygenases, which incorporate one oxygen atom into the substrate to form arene oxides; this is followed by the enzymatic addition of water to yield trans-dihydrodiols and phenols (Cerniglia, 1984). Bacteria characteristically produce dioxygenases, which incorporate two oxygen atoms into the substrate to form a dihydrodiol with a *cis*-configuration (Cerniglia, 1984). Further oxidation of *cis*-dihydrodiols leads to the formation of dihydroxy products that are substrates for another dioxygenase that brings about enzymatic fission of the aromatic ring (Wilson and Jones, 1993, Cerniglia, 1984). The initial ring oxidation is the rate-limiting step in the biodegradation reaction of polynuclear aromatic hydrocarbons (PAH) (Wilson and Jones, 1993). Common intermediate metabolites, namely, catechol, protocatechuic and gentisic acids are produced. These metabolites are then degraded by five similar pathways, which include ring cleavage, to produce succinic, fumaric, pyruvic and acetic acids and acetaldehyde. The remainder of the process to mineralization is similar to straight-chain hydrocarbons.

Co-metabolism or co-oxidation can be defined as microbial action that modifies chemical structure without yielding energy utilized for growth (King et al., 1992); it involves the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound and is caused by a relatively broad enzyme specificity on the part of the organism (Schneider and Billingsley, 1990). It is instrumental in the degradation of chlorinated aliphatics (Schneider and Billingsley, 1990) and has also been shown to be important in the degradation of cycloparaffins by

soil microorganisms (Frankenberger, 1992). Co-oxidation has also been observed to enhance the degradation of high-molecular-weight PAH i.e. those with four or more aromatic rings (Wilson and Jones, 1993).

2.2.2.5 Biodegradation Kinetics

The microbial breakdown of hydrocarbons in soils is often characterized by first-order kinetics (Frankenberger, 1992). First-order kinetics for oil decomposition at all concentrations have been used based on the conditions that nutrients are adequately supplied, oil is not added to exceed the immobile saturation and aeration is adequate (McGill, 1978). Many studies on petroleum hydrocarbon degradation have demonstrated the adequacy of first-order kinetics (Troy et al., 1994, Carberry, 1994). Specific studies on PAH biodegradation have demonstrated that first-order loss rates represented the data satisfactorily (Loehr, 1992, Park et al., 1990)

Michaelis-Menten kinetics have also been suggested for biodegradation kinetics i.e. zero-order for high substrate concentration and first-order for lower substrate concentration (Riser-Roberts, 1992). In most natural ecosystems, the numbers of hydrocarbon-utilizing microorganisms present will initially limit the rate of hydrocarbon degradation. But after a short period of exposure to petroleum pollutants, the numbers of hydrocarbon utilizers increase and will no longer be the principal rate-limiting factor. Verstraete et al. (1975) reported that in acclimatized soil biodegradation of gasoil proceeds more or less linearly with time i.e. zero-order kinetics.

Song et al. (1990) have noted that the kinetics of hydrocarbon degradation are complicated by the fact that there are numerous hydrocarbons within these oils which may be utilized at different rates. Other factors that can affect the shapes of substrate disappearance curves are (1) predation by protozoa, (2) time for induction of the active organisms, (3) accumulation of toxins produced by other microorganisms, (4) depletion of inorganic nutrients or other growth factors, (5) presence of other substrates that may repress utilization of the compound of interest and (6) binding of the compound to colloidal matter. These all contribute to the difficulty in predicting the kinetic of mineralization or disappearance of a particular substrate.

2.2.2.6 Biodegradation Products

In addition to carbon dioxide and water, the products resulting from complete mineralization of hydrocarbons, there are various fatty acids, hydroperoxides, alcohols, phenols, carbonyls, aldehydes, ketones and esters that result from incomplete oxidation (Riser-Roberts, 1992, Fan and Tanfuri, 1994). Intermediate metabolites of PAH biodegradation include dihydrodiols, phenols and arene oxides (Wilson and Jones, 1993). As mentioned earlier complete oxidation of hydrocarbons is more likely when a diverse mixture of microbes is available, avoiding the presence of by-products.

Some of the hydrocarbons may be incorporated into microbial cellular biomass. It is generally thought that 65% of the target hydrocarbon ends up as more cellular biomass and 35% is converted to carbon dioxide and water to supply energy requirements for cellular metabolism (McGill, 1978, King et al., 1992) while others state that only 33% of the hydrocarbons are converted to cell biomass and 66% to carbon dioxide (Fan and Tanfuri, 1994). More specifically, it has been reported that only 10% to 20% of the carbon of *n*-alkanes decomposed by soil organisms in 21 to 31 days was not accounted for as CO_2 (Frankenberger, 1992).

Via microbial biomass decay, and perhaps also more directly, some petroleum-carbon may become part of the soil humus. Through carbon dioxide evolution studies Bossert et al. (1984) have concluded that a major route of hydrocarbon disappearance is humification. Isotope studies have indicated that a considerable fraction of PAH compounds become incorporated into the humic component during bioremediation; while it is anticipated that the contaminants become essentially sequestered for an indefinite period of time after humic incorporation and thereby reduced in bioavailability, the possibility exists that portions of the incorporated fraction may become released (become unbound) at a later time (Piotrowski, 1991).

It is a common observation that the asphaltene portion of crude oils tends to increase rather than decrease during biodegradation, indicating that other hydrocarbon fractions are transformed to asphaltenes (Bossert and Bartha, 1984, Walker et al., 1976, Jobson et al., 1974). It is postulated that attack on hydrocarbons by oxygenases produces free radicals and other reactive intermediates that may chemically react with each other

forming partially oxygenated, cross-linked high-molecular-weight asphaltenes that are quite resistant to further biodegradation. It has also been recognized that some very long chain *n*-alkanes can be produced during biodegradation of petroleum (Oudot et al., 1989).

2.2.2.7 Effect of Bioremediation on Toxicity

The potential toxicity or inhibitory effect of petroleum hydrocarbons does not necessarily manifest itself in soils where biodegradation conditions are favorable. The most toxic components may volatilize or become immobilized by sorption to soil organic matter, although partial degradation of hydrocarbons may emulsify and release more harmful substances into the environment (Bossert and Bartha, 1984).

Wang and Bartha (1990) found that bioremediation treatment consisting of pH control, fertilization and tilling can restore soil contaminated by jet fuel, heating oil or diesel oil to a non-toxic condition as assessed by Microtox®, seed germination and plant growth. Wang et al. (1990) found that both mutagenicity and acute toxicity tests corroborated the effectiveness of bioremediation in destroying the PAH and other hazardous components of diesel oil (DO). Symons and Sims (1988) found that the degradation of individual PAH compounds in batch and soil column studies correlated with the decrease in Microtox® toxicity for all experimental conditions except for high loading rates where it was supposed that other organic constituents in the complex waste and soil mixture, which were not evaluated, contributed to the toxic response of the bioassay.

2.3 The Soil Environment

2.3.1 General

In this section, the soil environment is briefly described with respect to its physical, chemical and microbiological properties. Information on soil water is also presented. Finally the effects of different soil conditions on the bioremediation process are reviewed.

2.3.2 Soil Physical Properties

The soil environment consists, in various proportions, of gas, liquid and solid phases each of which may differ considerably in composition. As a consequence, the physico-chemical properties of soils vary greatly (Morgan and Watkinson, 1989). Soil solid materials range in size from stones to fine clays. The larger materials, called coarse or mineral fragments (including stones, cobbles, and gravels), are chemically and physically weathered over long periods of time to form the smaller soil particles of sand, silt and clay. The soil texture is defined by the relative proportion of sand, silt and clay in a soil sample.

Soil particles are held together by chemical and physical forces to form stable aggregates. The type of aggregates in a soil define its structure. Soil structure influences the amount of water that infiltrates a soil and gas diffusion at its surface; it also plays an important role in the movement of liquid and gaseous substances through soil as it defines its porosity. Soil structure is often improved with the addition of organic materials such as manures, sludges, composts and crop residues that are returned to the soil (Pierzynski, 1994).

Soil solid material is composed of minerals and organic matter. Soil minerals are classified as primary and secondary minerals on the basis of their origin. Primary minerals are those that are formed during the cooling of molten rock and are predominantly silicate minerals. The most important soil secondary minerals are the clay minerals due to their large surface area and reactivity with ionic and dissolved organic compounds. Clay minerals may bind protons, hydroxide ions, oxy-anions (particularly phosphate), metal cations, NH_4^+ and organic material, including microorganisms (Morgan and Watkinson, 1989). The bulk of the organic matter in soil is present as humus, a complex, relatively recalcitrant mixture of polymers produced by chemical and microbial attack on plant material.

2.3.3 Soil Chemical Properties

Major chemical properties of soils such as mineral solubility, soil reactions, pH, cation and anion exchange, buffering effects and nutrient availability are determined primarily by the nature and quantity of the clay minerals and organic matter present.

As mentioned above, layered aluminosilicate minerals, better known as clay minerals, have a profound influence on many soil chemical reactions because of their high active surface area. The term active refers to charges that develop on clay mineral surfaces and the ability of some types of clay minerals to expand.

Soil organic matter is comprised of decomposed plant and animal residues. It is a highly complex mixture of carbon compounds that also contains nitrogen, sulphur and phosphorous. It is made up of humic substances and biochemical compounds. Humic substances are operationally defined based on their solubility characteristics: humic acids are soluble in bases, but not acids; fulvic acids are soluble in acids and bases; and humin is the insoluble material that remains after humic and fulvic acid extraction (Pierzynski et al., 1994). Biochemical compounds include identifiable organic compounds such as organic acids, proteins, polysaccharides, sugars and lipids (Pierzynski et al., 1994). Soil organic matter can adsorb organic chemicals, in some cases enhancing both their biological and non-biological degradation (Pierzynski et al., 1994).

Because of their capacity for ion exchange, both clay minerals and organic matter can develop charged sites. Organic ions having charges that are opposite to the exchange site are attracted to the soil surface. In that manner, both clay minerals and organic matter have the ability to sorb soluble chemicals from the soil solution.

2.3.4 Soil Microbiological Properties

The relative abundance of organic substrates and attachment surfaces in the soil environment are both crucial factors that favor microbial abundance and diversity. Soil microorganisms include bacteria, fungi, algae and protozoa; although viruses can play an important part in the microbiology of soils they will not be discussed here. The two most abundant microbial groups are bacteria, including actinomycetes, and fungi. In

fertile soils, bacterial biomass may comprise 0.015-0.5% of the soil mass and fungal biomass may reach 0.05-0.5% (Bossert and Bartha, 1984) but together they only occupy only about 1 to 5% of the available pore volume in most soils (McGill, 1978). The high microbial biomass, the great microbial diversity and the abundant representation of bacterial and fungal genera capable of metabolizing hydrocarbons render soil a relatively favorable environment for petroleum biodegradation.

Bacteria are the most numerous of all soil microorganisms in soil (Paul and Clark, 1989). Estimates of bacterial numbers vary according to the means of determination. Plate counts usually give values ranging from several hundred thousand up to 200 million bacteria per gram of dry soil (10^5 to 10^8 range), the abundance being a reflection of the many environmental forces acting on these minute inhabitants (Alexander, 1977). Their ability to rapidly reproduce and adapt to new environmental situations is important to the decomposition and transformation of both natural and anthropogenic products. Some of the functions performed, either entirely or in part, by bacteria include: nutrient cycling, decomposition of organic materials, nitrogen fixation and oxidation-reduction reactions.

The fungi include eukaryotic organisms variously referred to as molds, mildews, rusts, smuts, yeasts, mushrooms and puffballs (Paul and Clark, 1989). Fungi perform several functions in soils including decomposing plant and animal organic substances and binding soil particles into aggregates (Pierzynski et al., 1994). In acid surface layers and forest soils, fungi make up the majority of the soil biomass and are most active in the decomposition process (Pierzynski et al., 1994).

2.3.5 Soil Water

Soils hold water in pore spaces by the cohesive and adhesive nature of water and soil particle surfaces. Cohesion forces are the result of water molecule polarity and hydrogen bonding, which attract water molecules to one another. Adhesion forces are responsible for attracting water molecules to soil mineral and organic matter surfaces. These forces allow water to move upward in soils by capillary action, or along surfaces of soil particles as water films.

Water moves in soils as a vapor or a liquid. Vapor flow through a soil is generally a slow process. Water vapor is present in all unsaturated soils and moves by diffusion within the soil due to vapor pressure and temperature gradients. Unsaturated water flow occurs whenever void spaces are partially filled with air.

2.3.6 Soil Conditions

2.3.6.1 General

In order for the biodegradation process to occur, soil conditions necessary to sustain microbial activity must be provided. The effects of soil moisture content, temperature, oxygen availability, nutrients, pH, soil type, organic carbon content and toxicity on the bioremediation process are reviewed.

2.3.6.2 Soil Moisture Content

The presence of oil reduces soil wettability but biological decomposition of the hydrocarbons will return the soil back to its normal wettability (Frankenberger, 1992). Water is necessary for microbial growth and the moisture content will also affect the diffusion of nutrients, substrate and by-products within the soil matrix; it is also important in determining the amount of available oxygen. The aerobic biodegradation of simple or complex organic material in soil is commonly greatest at 50 to 80% of the soil water-holding capacity (Riser-Roberts, 1992, Foght and Westlake, 1987, Frankenberger, 1992). Control methods that can be used are irrigation, drainage and landfarming.

2.3.6.3 Temperature

Petroleum biodegradation occurs at a wide range of soil temperatures. The soil temperature will affect the microbial growth rate and will also influence the soil moisture content. Freezing of the soil solution, of course, interrupts microbial activity (Bossert and Bartha, 1984). The optimum temperature for biodegradation of hydrocarbons ranges from 18 to 30°C (Frankenberger, 1992). Higher metabolic rates in response to elevated temperatures are balanced by the increased membrane toxicity of certain hydrocarbons at higher environmental temperatures. For outdoors facilities, soil temperature can be modified by the use of mulches of natural or artificial materials.

2.3.6.4 Oxygen Availability

The initial steps of aerobic hydrocarbon biodegradation are oxygen dependent (Bossert and Bartha, 1984); oxygen availability is directly related to soil moisture content as the pore space in the soil will be occupied by either water or air. Elimination of air-filled pore space, for instance, by waterlogging, reduces soil oxygen reserves to the small amount dissolved in the soil solution. The aforementioned moisture content range (50 to 80% holding capacity) also provides optimum aeration levels (Foght and Westlake, 1987). Two aspects of aeration are important. Oxygen must first penetrate the active soil layer to the necessary depth. It must secondly, at any depth, penetrate soil aggregates to reach the microorganisms. It has been shown that the penetration to depth is likely to be limiting only if soils are extremely active (Devinny and Islander, 1989). Penetration of soil aggregates, however, may be hindered if the aggregates are saturated with water or oil. The same control measures as are used to control soil moisture apply; as well as aiding in aeration and soil permeability, soil tillage is beneficial in increasing the probability of contact between substrate, nutrient and decomposer.

2.3.6.5 Nutrients

Inorganic nutrients are required for optimum biological growth; nitrogen, phosphorus, sulphur and potassium are important nutrients. Nitrogen is a key building block of proteins and nucleic acids while P is needed to produce enough ATP to carry out metabolic functions. While there is general agreement on the fact that a proper carbon : nitrogen : phosphorus (C:N:P) ratio is required for optimum microbial activity (Atlas, 1977; Atlas, 1981; Brown et al., 1983; Foght and Westlake, 1987; Sims, 1990; Autry and Ellis, 1992; Riser-Roberts, 1992; Rogers et al., 1993; Bandyiopadhyay et al., 1994; Carberry, 1994; Fan and Tafuri, 1994), there are varied and conflicting reports on the effects of adding inorganic nutrients to enhance soil bioremediation. The factors that appear to contribute to the varied responses in soils include the inherent nitrogen reserves of the soil, nitrogen fixation, and, last but not least, other overriding limitations such as temperature, oxygen, water, or pH that may have a more severe effect than the limitation by mineral nutrients (Bossert and Bartha, 1984). Furthermore, the effect of adding N and P is most obvious on the hydrocarbons that are structurally most

biodegradable; for example, studies have shown that addition of nitrogen or phosphorus stimulated degradation of saturated hydrocarbons more than of aromatic hydrocarbons (Riser-Roberts, 1992).

As the carbon, nitrogen and phosphorus content of a mixed microbial population in the soil is generally accepted to be in the ratio of 100 parts carbon to 10 parts nitrogen to 1 part phosphorus, the C:N:P ratio of the soil being bioremediated becomes the controlling parameter and optimum ratios reported in the literature range between 100:3:0.33 to 100:15:13 (Rogers et al., 1993, Riser-Roberts, 1992, Warith et al., 1992, Sims, 1990, Foght and Westlake, 1987, Zitrides, 1983). More generically, Frankenberger (1992) has stated that when the inorganic N content (NH_4^+-N and NO_3^--N) is maintained at >50 ppm, an adequate supply of N is usually available for biodegradation of oil; Fletcher (1994) reported maintaining soil nitrogen levels at 20 ppm and phosphorous levels at 10 ppm.

Theoretically, carbon utilization efficiency, organic nutrient mineralization and internal nutrient cycling through soil microbes and extracellular products should be taken into account when calculating inorganic nutrient requirements. Although McGill (1976) found total soil N and rate of remineralization of immobilized N to be important variables, the rate of oil decomposition and the amount of oil appeared to be more important in controlling N demand. This may be why in practice nutrient requirements are often determined on the basis of immediately available nitrate- and ammonia-nitrogen and orthophosphates levels (Warith et al., 1992, Flowers et al, 1984) and it is often assumed that the carbon content of hydrocarbon contamination is 85% (Riser-Roberts, 1992) and conversion efficiency is 60% (McGill, 1978).

Nitrogen used by microorganisms for growth is taken up in both the NH_4^+ and the NO_3^- forms; however Paul and Clark (1989) reported that many studies have indicated that NH_4^+, already being in the reduced state required for incorporation into amino acids, is preferred to NO_3^- and even low levels of NH_4^+ often repress the enzymes required for NO_3^- reduction. The amount of soil phosphorus available for uptake by living organisms is designated as available phosphorus and is the portion that is

extractable by dilute acid or bicarbonate (Paul and Clark, 1989). Thus measured available phosphorus is partly inorganic and partly organic.

Common inorganic fertilizers can be used to amend nutrient deficient soils and generally the best fertilizers for soil application are in a form of readily usable nitrogen and phosphorus or in a slow-release form to provide a continuous supply of nutrients that would not be leached from the oil-soil interface. It should be noted that nitrogen in the NO_3^- form is readily soluble in water and thus subject to leaching and water transport; in the NH_4^+ form it is subject to volatilization and to fixation both by clays and soil organic matter (Paul and Clark, 1989). Salinization due to excessive nutrient additions can be a serious problem (McGill, 1977) as high salinity can inhibit microbial activity. An added benefit to fertilizer addition is that the addition of P can improve the wetting characteristics of oily soils (Frankenberger, 1992).

In a study on the effect of organic nutrient addition, Dibble and Bartha (1979) did not find any stimulation of oil biodegradation in soil by either yeast extract or activated sewage sludge when applied in conjunction with inorganic fertilizers. These organic amendments actually suppressed oil biodegradation which could be attributed to competition for oxygen or repression of hydrocarbon-degrading enzymes by the availability of more readily utilizable substrates.

2.3.6.6 pH

Soil pH is related to the tolerance of microorganisms to specific pH ranges. It will also affect the solubility of macro-, especially phosphorus, and micro-nutrients making them more or less available to the organisms; the mobility of potentially toxic materials and the reactivity of minerals, with the potential for accumulation of acids, will also be affected by the soil pH. Soil pH will also affect the mobility of heavy metals. Most bacteria have limited tolerance for acidic conditions whereas fungi are more resistant. However, it has been shown that the highest rate of hydrocarbon biodegradation is attainable by a mixed bacterial-fungal community at neutral or slightly alkaline pH (7-8) (Bossert and Bartha, 1984). Lime can be used to control the pH in the treatment of oil-contaminated soils.

2.3.6.7 Soil Type

The type of soil being remediated will influence the moisture infiltration rate, permeability, water holding capacity and adsorption capacity for wastes. A predominance of clay and silt particles in finer textured soils results in a very small pore size, with a slow infiltration rate of water; mechanical tilling or the addition of exogenous materials (e.g. straw) can be used to enhance penetration of clays. However, tillage operations may be adversely affected by clay content in excess of 40% (McGill, 1978) but probably only if the soil moisture content is high. Coarse soils of sand and gravel have large interconnecting pores and allow rapid water movement; mechanical pre-processing can be used to increase uniformity in the soil matrix. The soil type also affects the types of microbial populations present (Riser-Roberts, 1992).

The chemical affinity between organic compounds and soil solids depends on structure (molecular weight, chain length, etc.) of the organic molecule, functional groups present in the organic molecule, configuration of the organic molecule and aqueous phase present (Kowalska et al., 1994). Adsorption of nonionic organic compounds, such as petroleum hydrocarbons, by clay soils is governed by the presence of C=O and C-N structures in those petroleum compounds. Molecules which possess many C=O or C-N groups adjacent to methylene groups would be more polar, and hence more strongly adsorbed than those compounds which possess fewer such groups. The nonpolarity of most petroleum hydrocarbons molecules permits only a weak interaction with the clay particle surfaces (van der Waals), with consequent lower levels of adsorption of the various hydrocarbons (Yong et al., 1992). It has been indicated that adsorption of hydrocarbon by clay surfaces occurs only when the solubility of the hydrocarbon is exceeded and the hydrocarbon exists in the micellar form (hydrocarbon-like regions in the water within which hydrocarbons preferentially dissolve) (Yong et al., 1992). It has also been stated that natural clays are ineffective sorbents for poorly water soluble, nonionic organic contaminants such as aromatic hydrocarbons (Kowalska et al., 1994). However, many hydrocarbon biodegradation intermediates contain C=O structures which could cause them to form stronger bonds with clay minerals.

2.3.6.8 Organic Carbon Content

The organic carbon content in the affected soil determines the existing biomass levels and nutrient reserves. The sorption of non-ionic organic contaminants, such as petroleum hydrocarbons, is mainly controlled by the organic fraction of the soil (Kowalska et al., 1994); it has been suggested that the organic carbon content of soil is the single most important factor determining the sorption of hydrophobic molecules such as PAH (Weissenfels et al., 1992). Adsorption occurs on the hydrophobic surfaces of the organic matter in the soil where nonpolar molecules are preferentially adsorbed over water (Yong et al., 1992). Some humic soils are known to retard biodegradation which may be due to the binding capacities of humic acids that may tie up certain nutrients and provide adsorption sites for hydrocarbon immobilization.

2.3.6.9 Toxicity

Short-chain paraffins below C_{10} generally are assumed to be toxic to microorganisms because of their relatively high water solubility and their interaction with membrane lipids (Wang and Bartha, 1994); although this effect might be compensated by their relatively high volatility, such compounds are known to affect the biodegradation process (Schneider and Billingsley, 1990). It should also be noted that the great absorption capacity of soil for both polar and nonpolar materials reduces the effective toxicity to microorganisms of all chemicals in soil.

Heavy crude and fuel oils contain potentially toxic trace metals which may accumulate in contaminated soils. It has been noted that the heavy metals Mn, Cu, Pb, Cr and Zn accumulated in the top 30 cm when 5000 ppm oily waste was applied to a neutral soil (Bossert and Bartha, 1984); these heavy metals are more readily mobilized in acidic soils and therefore maintaining a neutral to slightly alkaline soil will provide for heavy metal immobilization. Toxicity to microorganisms might arise during the remediation process due to the presence of heavy metals such as lead, mercury, cadmium, chromium and nickel. Frankenberger found that presence of Pb to a concentration of up to 1000 ppm did not have an effect on mineralization of diesel fuel in terms of cumulative amount of CO_2 produced; the same was found for concentrations of Cd of up to 100 ppm (Frankenberger, 1992). In cases of high heavy metal

contamination it may be possible to treat with a chelating agent before treatment (Schneider and Billingsley, 1990).

2.4 Contaminant Characterization

2.4.1 General

In this section, contaminant characteristics are reviewed. First, a general overview of the petroleum products carried by IPL is given. Secondly, relevant physico-chemical properties of petroleum hydrocarbons are reviewed. Thirdly, the concept of contaminant bioavailability is described as it relates to the physico-chemical properties of hydrocarbons. Fourthly, information on the biodegradability of different types of petroleum hydrocarbons is reviewed. Information on the biodegradability of crude oil and other petroleum products is also presented as they represent complex hydrocarbon mixtures. Finally, susceptibility of hydrocarbons to abiotic transformation processes within the soil environment is reviewed.

2.4.2 Overview

The IPL/Lakehead Pipe Line Company system carries roughly 65 individual types of crude oils belonging to five general classes (Aspen Research Corporation, not dated):

a) light: the lowest viscosity class which represents approximately 50% of IPL/LPL deliveries;

b) medium: moderate viscosity which is frequently considered with heavy;

c) heavy: high viscosity oil which, along with medium, accounts for roughly 35% of deliveries;

d) synthetic: synthetic crude oil material which represents a small percentage of IPL/LPL deliveries; and

e) condensate: similar to light which represents a small relative percentage of deliveries.

Crude oils are extremely complex mixtures, possessing hundreds of individual compounds; because of the wide variation in crude oils, it is improbable that a

comprehensive, compound-by-compound analysis will ever be conducted (Aspen Research Corporation, not dated). Though crude oil primarily contains hydrocarbons, many other minor, yet important, constituents are commonly present; sulfur, nitrogen, and oxygen are all present within various crude oil compounds. The results of a crude oil characterization undertaken by Aspen Research Corporation (Aspen Research Corporation, not dated) on four crude oils representative of those carried within the IPL system showed that hydrocarbon compounds made up approximately 92 percent of these crude oils; the sulfur content varied from 3.5% to less than 0.5%. The nitrogen content was less than 0.25%. Trace levels of a variety of heavy metals were also present.

On a structural basis, the hydrocarbons in crude oil are classified as alkanes (normal and branched), cycloalkanes and aromatics; alkenes are rare in crude petroleum. Increasing carbon numbers of alkanes, variations in carbon chain branching, ring condensations and interclass combinations account for the enormous numbers of hydrocarbons that occur in crude petroleum (Bartha, 1986). The smaller amounts of high molecular weight oxygen-, nitrogen- and sulfur-containing compounds are collectively designated as "resins" or "asphaltenes" (Shell International Ltd., 1983).

Refined products are also transported by IPL Inc. and these products can be composed of as many as several hundred constituents (American Petroleum Institute, 1993). Similarly to crude oils, the chemical constituents of petroleum products can generally be categorized into three different groups: alkanes, cycloalkanes and aromatics. Alkanes are linear hydrocarbon chains characterized by single chemical bonds between carbon atoms. They can be straight chains, branched chains or ring structures. Cycloalkanes are hydrocarbon rings joined by single carbon-carbon bonds. Aromatic compounds contain at least one benzene ring.

Fractionation products derived from crude oil, such as diesel petroleum, fuel oil, lubricating oil, etc., contain PAH. The crude-oil source and fractionation process used have an effect on the PAH content of the final fuel products, and higher concentrations of PAH are associated with the higher boiling-point distillation products.

2.4.3 Physico-Chemical Characteristics

2.4.3.1 General

The physico-chemical characteristics of the contaminants influence their prevalence in the different soil phases (gas, water, inorganic solids, organic solids) and hence their availability to biodegradation processes; they also define their susceptibility to removal from the soil system by other physical processes. As stated earlier, crude oils and petroleum products can be composed of many hundred different constituents and the extent to which these constituents partition among soil phases depends on their individual properties.

Aliphatic and aromatic hydrocarbons are neutral nonpolar organic liquids. The nature of the functional groups which form the compound will influence its characteristics and its ability to bind with the soil constituents. Depending on how they are placed, the functional groups will influence the characteristics of organic compounds, and will thus contribute greatly in the determination of the fate of these compounds in soil (Yong et al., 1992).

In this section the different hydrocarbon types described above will be broadly defined on the basis of their water solubility, soil/water partition coefficient and volatility.

2.4.3.2 Water Solubility

Solubility of contaminants in water is important as the degradation process is thought to depend mainly on the availability of nutrients and contaminants in the water phase. Substances which are more soluble are more likely to desorb from soils and less likely to volatilize from water.

Organic compounds differ widely in their solubility from infinitely miscible polar compounds to extremely low solubility nonpolar compounds, such as high molecular weight PAH. The water solubility of liquid hydrocarbons decreases with increasing molecular weight within each hydrocarbon class; branching of hydrocarbon isomers and condensed ring formation tends to increase their solubility in water (Frankenberger, 1992). Water solubility at 25°C of some hydrocarbon constituents have been shown to

vary from 0.005 mg/L for dodecane to 1780 mg/L for benzene (American Petroleum Institute, 1993).

The octanol/water partition coefficient (K_{ow}) can also be used as a measure of a compound relative prevalence in water as it represents the distribution of a chemical between water and an organic phase; it is well correlated with the solubility of several organic chemicals (Yong et al., 1992).

Emulsifiers can be employed to increase the surface area of low solubility compounds making them more bioavailable. It has been found that some organisms produce their own emulsifiers (Riser-Roberts, 1992, Bossert and Bartha, 1984). The release of CO_2 by hydrocarbon oxidizers also tends to emulsify the oil. Other microbial emulsifying agents include organic acids and long chain fatty acids which increase the interface for microbial utilization of the water insoluble components of the oil (Frankenberger, 1992). The application of nutrients may enhance the bio-emulsifying activity of microorganisms (Frankenberger, 1992). The solubilization of hydrocarbons in soil is also said to be promoted through microbial activity due to the fact that oxygenated hydrocarbon metabolites become more soluble (Bossert and Bartha, 1984).

The soil environment also affects hydrocarbon solubility. It has been shown that by their molecular, sieve-like nature, fulvic acids retain hydrophobic compounds on their surface and within their structures, thereby rendering such hydrophobic compounds more soluble (Bossert and Bartha, 1984, Robinson, 1994). Conversely, water-insoluble humic acids retain and thus immobilize hydrophobic compounds.

2.4.3.3 Soil/Water Partition Coefficient

The soil/water partition coefficient (K_{oc}) is essentially a coefficient that describes the distribution of the organic chemical between aqueous and soil organic matter phases. Sorption is perhaps the most important single factor affecting the behavior of organic chemicals in the soil environment (Pierzynski et al., 1994). Adsorption to soil constituents will affect the rate of volatilization and diffusion as well as the availability of chemicals to microbial or chemical degradation. Sorption/desorption reactions may be rate limiting; it has been shown that adsorbed organic material is less available to

bacterial degradation than the same material in a dissolved state and the decreased availability is a function of the degree of adsorption (Tabak et al., 1994).

Values for K_{oc} are not commonly reported in the literature, but they can be measured experimentally or estimated on the basis of K_{ow}. K_{oc} values have been empirically derived for some hydrocarbon constituents and can vary from 190 L/kg for benzene to 88 000 L/kg for dodecane (American Petroleum Institute, 1993). PAH are relatively insoluble in water and are therefore associated primarily with the organic matter (Wilson and Jones, 1993).

2.4.3.4 Volatility

Volatilization of organic chemicals is responsible for the transfer of organic chemicals from soil environments into the atmosphere; it is important to consider in the bioremediation process as it may represent a significant pathway for removal of some volatile hydrocarbons. The volatility of a compound can be defined by its vapor pressure and solubility (Henry's Law constant) and adsorption-desorption characteristics. The soil moisture content, porosity, density, organic matter and clay contents, and environmental factors such as temperature, humidity and wind speed will influence the rate of volatilization.

It has been stated that up to 20 to 40% of crude oils may volatilize from soil while other field studies showed that less than 0.1% of a crude oil evaporated from soil (Bossert and Bartha, 1984). Generally the volatility of alkanes decreases as the length of the chain increases and *n*-alkanes greater than C_{18} do not exhibit significant volatilization at ambient temperatures. Park et al. (1990) showed that volatilization loss was important for some two ring-PAHs. It is also thought that biodegradation and evaporation compete in the removal of petroleum hydrocarbon (Song et al., 1990).

2.4.4 Bioavailability

It has been stated that the physical and chemical properties that will most directly affect the effectiveness of bioremediation of petroleum products are bioavailability and microbial degradability of the individual constituents (Fan and Tafuri, 1994). The concept of bioavailability is explained separately here as it brings together different ideas

explained above; information on susceptibility to microbial degradability is presented in a subsequent section.

Organic compounds may be present in different phases of the soil environment i.e. free in solution, bound to dissolved organic matter, bound to a mineral surface or bound to soil organic matter. Bioavailability is not only related to the partition of a compound among those phases in the soil, but the kinetics of the adsorption and desorption processes are also of importance (Harmsen, 1991, Tabak et al., 1994). Adsorption occurs when solute molecules are driven from solution, at concentrations below maximum solubility, thereby allowing a more thermodynamically favorable state. Although partitioning has been attributed as the major mechanism for adsorption of nonionic chemicals, it is not the only interaction that may occur; soil surfaces are heterogeneous and many types of interactions may control adsorption on them (Robinson, 1994).

Kinetically, sorption is a two-phase process, with an initial fast stage (< 1 hour) followed by a slower long phase (days) (Tabak et al., 1994). The initial fast adsorption process is thought to reflect rapid adsorption of the hydrophobic compounds onto hydrophobic areas of soil surfaces, whereas the following slow adsorption process is proposed to be based on migration of the hydrophobic contaminants to less accessible sites within the soil matrix (Weissenfels et al., 1992). Once adsorbed by surface organic matter, an organic contaminant may be easily desorbed, desorbed with difficulty or not desorbed at all. The process by which contaminants desorb from soil is only partially understood (Robinson, 1994).

Early researchers stated that oil decomposition in soil occurred at the oil-water interface and that it was probable that most oily substrates moved to microbes as emulsion or in solution (McGill, 1978). More recently, it has been reported that liquid hydrocarbons can be taken up and incorporated into cell membranes (Robinson, 1994); however the mechanisms of utilization of sorbed substrates are not fully understood. Some of the high molecular weight hydrocarbons which are insoluble in water are attacked by microbes growing at the oil-water interface; emulsification plays an extremely important role in increasing this interface (Frankenberger, 1992). Solid- and

slurry-phase soil bioremediation experiments involving different crude oils and refined petroleum products found that fractions of high molecular weight ($>C_{44}$) saturates and aromatics were biodegraded (Huesemann, 1995). However it has also been suggested that PAH are used only in the dissolved state (Robinson, 1994) but one study has also found that phenanthrene was mineralized even when all was sorbed (Manilal and Alexander, 1991). It is thought that microorganisms may use a water-insoluble substrate as it spontaneously dissolves in water, or they may metabolize the compound after a biologically mediated solubilization or by mechanisms involving physical contact with the insoluble phase of the substrate. A study has shown that mineralization of octadecane was about 200 times faster than its spontaneous dissolution, so that the microorganisms were either acting on the insoluble compound or enhancing its dissolution (Thomas et al., 1986).

It has been reported that organic compounds that complex with humic colloids were protected from subsequent microbial decomposition (Robinson, 1994, Tabak et al., 1994). The effect of sorption on PAH bioavailability was demonstrated by the reduction of PAH-degradation rates with increasing sorption capacity of the sorptive substrates used (Weissenfels et al., 1992). It then follows that if a microorganism cannot use the adsorbed form of a chemical, it may be expected that the organisms will first metabolize the chemical that is in solution and that the subsequent rate of transformation of the sorbed compound will be limited by the rate of desorption. It has been stated that removal of the substrate from the surface of the soil is the rate limiting step (Tabak et al., 1994).

At this point it is not known whether sorption alone renders a compound unavailable for uptake by microbes (Robinson, 1994). It is possible that bacteria and organic contaminants may be sorbed on adjacent locations on the soil surface, hence facilitating scavenging of the compound by the sorbed bacteria.

It can be stated that in interpreting the effect of soil surfaces on bioconversion processes, all possible physical or chemical interactions (diffusion, adsorption, desorption, ion-exchange reactions, etc.) of a given compound and its possible

metabolites with a given surface have to be considered before general conclusions can be drawn (Tabak et al., 1994).

2.4.5 Biodegradability of petroleum hydrocarbons

2.4.5.1 General

The chemical structure of a compound will affect its susceptibility to biodegradation in two ways. First, the molecule may contain groups or substituents that cannot react with available or inducible enzymes (i.e., these chemical bonds cannot be broken). Second, the structure may determine the compound to be in a physical state (adsorbed, gas-phase) where microbial degradation does not easily occur; the concept of bioavailability has already been discussed.

In this section information on biodegradability of *n*-alkanes, alkylaromatics, aromatics, branched alkanes, cycloalkanes and other components of petroleum products is presented.

2.4.5.2 n-Alkanes, alkylaromatic and aromatic hydrocarbons

The least toxic and most readily biodegradable petroleum components are the *n*-alkanes, alkylaromatics, and aromatic compounds of the C_{10} - C_{22} range. *n*-Alkanes, alkylaromatic, and aromatic hydrocarbons in the C_5 - C_9 range have relatively high solvent-type membrane toxicity but in most environments they are removed by volatilization rather than by biodegradation (Bossert and Bartha, 1984). Those compounds above C_{22} have low toxicity, but their extremely low water solubility and their solid state at physiological temperatures make them unfavorable for biodegradation.

PAH biodegradability is generally expressed on the basis of ring number. PAH persistence has been shown to increase with molecular weight or compound ring number; two- and three-ring PAH compounds have been found to be extensively bio-transformed in soil systems whereas PAH compounds with more than three rings were more persistent (Park et al., 1990) It has been reported that PAH biodegradation correlated positively with water solubility rather than with the degree of condensation

(cluster against linear arrangement of the same number of rings) (Wilson and Jones, 1993). It is to be noted that because of their chemical stability the transformation of PAH in soil is chiefly microbial (Manilal and Alexander, 1991, Weissenfels et al., 1992).

2.4.5.3 Branched Alkanes and Cycloalkanes

Branched alkanes and cycloalkanes in the C_{10} - C_{22} range are less biodegradable than their *n*-alkane and aromatic analogs. Branching creates tertiary and quaternary carbon atoms that constitute a hindrance to β-oxidation (Bossert and Bartha, 1984). The biodegradation of cycloalkanes requires synergistic cooperation of two or more microbial species, and cycloalkanes of C_{10} and below have high solvent-type membrane toxicity (Bossert and Bartha, 1984). Cycloparaffinic systems with four or more condensed rings are mostly resistant to biodegradation (Bossert and Bartha, 1984).

2.4.5.4 Others

The partially oxygenated and condensed components of tar, bitumen and asphalt are also mostly resistant to biodegradation. Sulphur-containing heterocycles from Prudhoe Bay crude oil have been shown to be susceptible to microbial degradation under laboratory conditions (Fedorak and Westlake, 1984a). Fedorak and Westlake (1984b) also showed the susceptibility of some C_1-, C_2-, and C_3- and one C_4-carbazole isomers from Norman Wells crude oil to biodegradation by a carbazole-enriched culture under laboratory conditions. It has also been reported that aromatic nuclei containing sulphur were twice as refractory as non-sulfur analogs (Walker et al., 1976).

2.4.6 Biodegradability of Crude Oil and Other Petroleum Products

2.4.6.1 General

Based on the information presented above, biodegradability of complex mixtures of different hydrocarbon types such as crude oil and other petroleum products is reviewed.

2.4.6.2 Crude Oil

Some components of oil will never likely be subject to very rapid metabolisms since they already closely resemble soil humus. The amount of oil subject to some consequential amount of microbial degradation probably never exceeds 80% of the oil added to soil and may be as low as 30 to 40% where very large volatile losses occur or where a crude oil is extremely heavy and asphaltic (McGill, 1976). It has also been stated that as little as 11% of some "heavy" asphaltic-naphthenic crude oils may be biodegradable within a reasonable time period, even if conditions are favorable (Bartha, 1986). Walker et al. (1976) found that 82% of a South Louisiana crude oil was degraded in a flask biodegradability study. From his studies and literature reviews McGill (1976) estimated the probable maximum rate of microbial decomposition at 0.7 yr^{-1} ($t_{1/2}$=1 year) for saturates or paraffinic oils and the minimum rate at about 0.05 yr^{-1} ($t_{1/2}$=14 years) for asphaltic oils or for the NSO fractions of oil.

2.4.6.3 Other Petroleum Products

Petroleum fuel products are usually classified as low-boiling point distillates such as gasolines, middle distillates such as diesel fuels, heating oil (no. 2 fuel oil), kerosene and jet fuels and high-boiling point distillates such as bunker C (residual fuel oil or no. 6 fuel oil) (American Petroleum Institute, 1993; Song et al., 1990).

Through column studies, Song et al. (1990) have demonstrated that bioremediation has only very limited beneficial effects on gasoline and bunker C elimination from soil; the C_6 to C_9 components of gasoline were lost more rapidly by evaporation than by biodegradation which primarily removed the C_{10} to C_{11} components while most bunker C components were deemed to be structurally resistant to biodegradation. Walker et al. (1976) found that only 11% of a bunker C oil could be degraded in flasks biodegradability studies. Environmental persistence of medium distillate fuels increases in the following order: jet fuel < heating oil < diesel oil (Song et al., 1990). Kerosene that consists almost exclusively of medium chain length alkanes is, under suitable conditions, 100% biodegradable (Bartha, 1986).

2.4.7 Abiotic Transformation Processes

The literature conspicuously lacks reports on the abiotic oxidative transformations of hydrocarbons in terrestrial environments, apparently reflecting the minimal significance of this process in soil (Bossert and Bartha, 1984). Thermal degradation of hydrocarbons is negligible at environmental temperatures below 80°C. Photolysis may also play a role in the degradation of organic chemicals at soil surfaces (Pierzynski et al., 1994) although penetration of oil below the soil surface limits oxygen availability and exposure to solar radiation. The oxygenated organic products of photooxidation are more water-soluble than the parent hydrocarbons and usually exhibit greater toxicity to the microbiota (Bossert and Bartha, 1984). In that manner photooxidation may also render some of the high molecular weight hydrocarbons to become more susceptible to biodegradation (Frankenberger, 1992).

2.5 Soil Remediation Applications

2.5.1 General

Information on relevant soil cleanup criteria in jurisdictions across North America and a review of petroleum contaminated soil bioremediation applications are presented here.

2.5.2 Soil Cleanup Criteria

Table 1 summarizes soil cleanup criteria for hydrocarbon contaminated soils used in different jurisdictions. Information was compiled from ASTM Data Series 64 publication (ASTM, 1995). In some cases specific references were included by the editor and they are provided here. To provide a basis for comparison with the cleanup criterion specified in this study, values were only reported where a broad parameter such as, or similar to "Total Petroleum Hydrocarbons (TPH)" was used. Although it must be kept in mind that some criteria were defined on a site-specific basis, it can be seen that numerical values vary widely, ranging from 10 ppmw to 20 000 ppmw. Some jurisdictions also differentiate on the basis of the type of petroleum product, for example, setting different criteria for gasoline vs diesel contamination. This summary also

highlights the fact that only a few jurisdictions specify an analytical method. This is a significant deficiency as it has been shown that different analytical methods will yield different estimates of petroleum concentrations (Huesemann, 1995). Another deficiency is the lack of compliance assessment standards. Such a standard was reported only in one jurisdiction.

Some jurisdictions also regulate for "Total Polycyclic Aromatic Hydrocarbons". The jurisdictions that do not utilize some type of "Total Petroleum Hydrocarbons" parameter have remediation values for specific compounds such as naphthalene, phenanthrene, etc. which utilizes a health risk assessment approach to regulate specific compounds known and/or suspected to present a risk.

The information presented above highlights the need for further research into the development of the methodology required to establish standardized soil cleanup criteria with respect to petroleum contamination. Such cleanup criteria should also define appropriate analytical methods and compliance assessment standards.

Table 1. Petroleum-Contaminated Soil Cleanup Criteria.

Type	Parameter	Numerical Value	Ref.
Soil Cleanup Guideline	Total Hydrocarbons (for pH > 6.5)	20 000 ppmw	The Development of Soil Cleanup Criteria in Canada, vol. 1 CCME-TS/WM-TRE015, IP-105, March 1990
Soil cleanup guideline (based on phytoxicity) Commercial/ Industrial Soil	oil and grease (fresh) (weathered)	10 000 ppmw 20 000 ppmw	Richardson, G.M., "Inventory of Cleanup Criteria and Methods to Select Criteria," Unpublished Report, Committee on Industrial Site Decommissioning, Industrial Programs Branch, Environment Canada, Ottawa, Ontario K1A 1G2. 46 pages, 1987.
Action level, guideline (Arkansas)	TPH	100 ppmw	
Site-specific health-risk-based approach (Delaware)	TPH -gasoline-contaminated soil -diesel-contaminated soil -waste oil-contaminated soil	 ≤ 100 ppmw ≤ 1000 ppmw ≤ 1000 ppmw	
Guidelines for leaking underground storage tank sites (District of Columbia)	TPH	100 ppmw	
Site-specific cleanup levels (California)	TPH	10 to 10 000 ppmw	

Table 1. Petroleum-Contaminated Soil Cleanup Criteria Continued.

Type	Parameter	Numerical Value	Ref.
Guidelines for Assessment and Remediation of Petroleum Contaminated Soil (Florida)	Total Recoverable Petroleum Hydrocarbons (TRPH) (Test Method 3540/9073)	10/50 ppmw *concurrent limit for PAH and Volatile Hydrocarbons	DER Guidelines for Assessment and Remediation of Petroleum Contaminated Soil, May 1992.
Site specific cleanup criteria for petroleum hydrocarbon contamination (Georgia)	TPH	100 to 500 ppmw	
Site-specific cleanup levels (Idaho)	TPH -gasoline contamination -diesel contamination -waste oil contamination	 40 to 200 ppmw 100 to 2000 ppmw 100 ppmw	
Site-specific, case-by-case basis (Indiana)	TPH gasoline, kerosene, naptha and diesel contamination	≤ 100 ppmw (on-site)	
General cleanup standard for petroleum-contaminated soils (Kentucky)	TPH	10.0 ppmw (detection limit)	
Interim soil cleanup standards (Kansas)	TPH	100 ppmw	Department of Health and Environment, Bureau of Environmental Remediation, Interim Soil Cleanup Standards, August 1993.
Site-specific action levels (Iowa)	TPH	100 ppmw	

Table 1. Petroleum-Contaminated Soil Cleanup Criteria Continued.

Type	Parameter	Numerical Value	Ref.
Typical cleanup level for petroleum-contaminated soil (Louisiana)	TPH	300 ppmw	
Site-specific risk-based standards (Massachusetts)	TPH	5000 ppmw	310 CMR 40.09-40.09.33, Amended 19 Nov. 1993 (MA Contingency Plan)
Typical cleanup level (Mississippi)	TPH	100 ppmw	
Typical soil cleanup levels for petroleum contamination (site specific) (Missouri)	TPH	max 500 ppmw	
Cleanup guidelines for petroleum releases (Montana)	TPH	100 ppmw *may be higher for diesel or other heavier fuels	Cleanup Guidelines Applicable for Petroleum Releases under the Montana UST Program, May 1992.
Site specific petroleum contaminated soil cleanup levels (Nebraska)	TRPH	10 to 500 ppmw	
Remediation goals (New Hampshire)	TPH -gasoline -all virgin petroleum product except gasoline	 10 ppmw 100 ppmw	
Cleanup standards for contaminated sites (New Jersey)	TPH -residential-direct contact and impact to groundwater soil	 10 000 ppmw	
Remediation Policy (Nevada)	TPH	100 ppmw (measured using EPA Method 8015 modified for petroleum hydrocarbons)	NDEP Contaminated Soil and Groundwater Remediation Policy, 25 June 1992.

Table 1. Petroleum-Contaminated Soil Cleanup Criteria Continued.

Type	Parameter	Numerical Value	Ref.
Cleanup guideline (New Mexico)	TPH	100 ppmw	
Proposed Regulation for cleanup levels for petroleum-contaminated sites - site specific (North Carolina)	TPH -low boiling point hydrocarbons -high boiling point hydrocarbons	 10 to 300 ppmw (EPA Method 5030) 40 to 200 ppmw (EPA Method 3550)	
Guidance to cleanup requirements - site-by-site basis for UST-petroleum contamination (North Dakota)	TPH	100 ppmw	
Cleanup guidelines -health risk based (Ohio)	TPH -gasoline -diesel and waste oil	 105 to 600 ppmw 380 to 1156 ppmw	Ohio EPA Policy DERR-00-RR-009, How Clean is Clean?, 26 July 1991.
Cleanup regulation - Remediation Index used in determining cleanup standards on a site-by-site basis (Oklahoma)	TPH	50 to 1000 ppmw	
Soil cleanup guidance levels for petroleum contaminated soils (Pennsylvania)	TPH	10 ppmw	
Guidance levels - site specific stds can be requested (TE)	TPH (for industrial area)	250 ppmw	
Guideline for remediation or removal of petroleum-contaminated soils (South Dakota)	TPH	10 to 100 ppmw (depending on proximity to aquifer, soil permeability, etc) CA/USGS method	

Table 1. Petroleum-Contaminated Soil Cleanup Criteria Continued.

Type	Parameter	Numerical Value	Ref.
Cleanup levels - site specific basis - Action level used as a starting point (Utah)	TPH TRPH oil and grease	30 - 10 ppmw 100 ppmw 300 ppmw	
Site specific cleanup levels-industrial area (Washington)	TPH -gasoline contamination -other	 100 ppmw 200 ppmw	
Site specific soil cleanup levels - action level (West Virginia)	TPH -gasoline contamination -diesel contamination	 50 ppmw 100 ppmw	

2.5.3 Selected Results From Literature

Table 2 summarizes a review of laboratory experiments and field operations which studied petroleum hydrocarbon biodegradation. This review focused on the degradation of petroleum products, as opposed to single compounds, where bioremediation treatment made use of indigenous soil microorganisms and included tilling, soil pH control and inorganic nutrient addition. Experimental conditions were also differentiated on the basis of whether the contaminant was added to the soil for the purpose of experimentation only (contaminant added (CA)) or whether contaminated soil was collected for the experiment (contaminated soil (CS)).

It is to be noted that in field experiments it is usually not possible to distinguish between oil removal by biodegradation vs abiotic mechanisms such as evaporation, runoff and leaching. There are also difficulties in even application of the contaminant and representative sampling are also inherent to field experiments. Temperature, precipitation and other such factors vary and influence the results in unpredictable ways. These difficulties need to be taken into account when field experiment data are evaluated and compared. There are also difficulties arising from the fact that different analytical procedures are used and that some analytical procedures are not well standardized.

Table 2. Selected Results from Petroleum-Contaminated Soil Biodegradation Studies.

Contaminant	Experimental Conditions	Analytical Method	Initial Concentration	Time	Reduction	Reference
Bachaquero gasoil	20-L boxes contaminant added (CA)	CCl_4 extraction & gravimetric quantification	5000 ppm	42 d	40.6% Total Fatty Matter (TFM)	Verstraete and Vanloocke, 1975
Nigeria gasoil	"	"	"	"	64.3% TFM	"
Kuwait gasoil	"	"	"	"	76.6% TFM	"
drilling cuttings containing 66% fuel oil	100-cm^3 beakers contaminated soil (CS)	CCl_4 extraction & GC quantification	2670 ppm	60 d 270 d	50% total HC 75% total HC	Chaineau et al., 1995
no. 5 fuel oil	above-ground treatment cell (CS)	SM 503D & E	1300 to 5000 ppm	40 d 180 d	79% TPH 88% TPH	Heely et al., 1994
jet fuel	glass columns (22 mm dia., 150 mm length) (CA)	CH_2Cl_2 extraction & GC quantification	50 000 ppm	84 d	60 % total HC	Song et al., 1990
no.2 fuel oil	"	"	135 000 ppm	63 d	50% total HC	"
diesel oil	"	"	100 000 ppm	126 d	55% total HC	"
no.6 fuel oil	"	CH_2Cl_2 extraction & gravimetric quantification	50 000 ppm	336 d	negligible	"
California crude oil	field plots (CS)	EPA 8015 M (C_7-C_{30})	4000 ppm	40 d	75% TPH	Ju et al., 1993

Table 2. Selected Results from Petroleum-Contaminated Soil Biodegradation Studies Continued.

Contaminant	Conditions	Analytical Method	Initial Concentration	Time Frame	Reduction	Reference
Arabian heavy crude oil	field plots (CA)	benzene extraction & gravimetric quantification	19 000 ppm to 31 000 ppm	365 d	71% total oil concentration (OC)	Raymond et al., 1976
Gulf Coast Mix crude oil	"	"	"	"	62% total OC	"
no.2 fuel oil	"	"	"	"	92% total OC	"
no.6 fuel oil	"	"	"	"	52% total OC	"
kerosene	field plots (CS)	*n*-hexane extraction & GLC quantification	8700 ppm	630 d	100 % oil	Dibble and Bartha, 1979
diesel oil	486-L outdoor boxes (CA)	CH_2Cl_2 extraction & GC quantification	60 000 ppm	84 d 140 d	83% total HC 95% total HC	Wang et al., 1990

3.0 Field Work

3.1 Introduction

As highlighted in section 1.3, in order to gain a better understanding of operating conditions and practices necessary to optimize performance at IPL's RBFs, a specific objective of this research project was to conduct soil monitoring activities at the Edmonton RBF. To accomplish this objective a soil monitoring program was developed and is detailed below. Analytical methods used are also described in this section. Finally, soil monitoring results are presented and discussed.

3.2 Monitoring Protocol

3.2.1 General

Three major sampling events were conducted at the Edmonton RBF between May and October 1995. Organic and inorganic parameters were assessed at the beginning of the season for all three cells and in October for Cells C and D as new soils were placed in those cells in September 1995. Only organic parameters and major ions were analyzed in July for all three cells and in October for Cell A. In addition, Cells C and D were sampled for T.E.H. and available nutrients in September.

3.2.2 Analytes of Interest

Analytes of interest are listed in Table 3. The decision to assess hydrocarbon content as T.E.H. (C_7 - C_{30}) vs T.E.H. (C_7 - C_{60}) as in 1994 was made by IPL environmental staff. It was based on the perceived low risk, in a industrial end-use context, associated with the presence of higher molecular weight compounds which are less susceptible to environmental transformation or removal mechanisms such as volatilization and water solubilization.

Phenols contents were analyzed as part of the regulatory requirements and also based on the fact that some crude and refined oils contain considerable amounts of phenols that may contribute to the toxicity of oil (Sauer and Boehm, 1991). Metals,

routine soil quality, available nutrients, moisture content, particle size distribution and microbial enumeration analyses, THB, were conducted to assess suitability of soil conditions for the bioremediation process. Some metals and other parameters assessed under routine soil quality were also considered as part of the regulatory requirements. Microtox® analyses were conducted to provide information on the relative toxicity of the water soluble fraction (WSF) of the soil contaminants as a function of time.

3.2.3 Sampling Times

Sampling times are included in Table 3.

Table 3. Analytes of Interest and Sampling Times-Field Work 1995.

Analyte	Sample Type	Frequency
T.E.H. (C_{10}-C_{30})	grab and composite	May, July, Sep (Cells C and D only), Oct
Phenols	grab	May, July, Oct
Metals	composite	May, Oct (Cells C and D only)
Routine Soil Quality	composite	May, July, Oct
Available Nutrients	composite	May, July, Sep (Cells C and D only), Oct
Moisture Content	grab and composite	May, July, Oct
Particle Size Distribution	composite	May, Oct (Cells C and D only)
Microtox®	composite	May, July, Oct
Microbial Enumeration (THB)	composite	May, July, Oct

Routine soil quality includes total organic carbon(TOC) and detailed salinity (Ca^{2+}, Cl^-, electrical conductivity (EC), K^+, Mg^{2+}, Na^{2+}, pH, sodium adsorption ratio (SAR), % saturation and SO_4^{2-}).

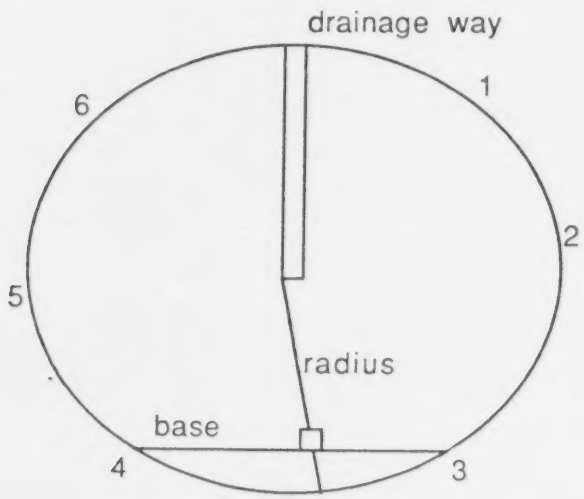
Available nutrients include ammonia-N, nitrate-N and orthophosphates.

3.2.4 Sampling Points

A random sampling technique was used to collect discrete samples from the cells. Each circular treatment cell was divided into six equal sectors with an arc of 60° and one grab sample was taken from each sector. This number of samples was judged to be valid as a review of the 1994 monitoring data showed that the standard error of the cell mean ranged between 9% and 22% (for T.E.H.) which was considered acceptable.

The sampling point within each sector was determined by randomly choosing a distance between 0.0 and 30.5 meters measured from the center of the cell along the radius and between 0.0 and 30.5 meters measured on a straight line between the permanent metal T-bars installed in 1994; see Figure 1 for illustration of sampling point location. This sampling scheme assumed that all cells had the same dimensions and were a perfect circle; adjustments were made on site in the cases where these assumptions did not hold. The grab samples actually consisted of a composite of four plugs taken at the four extremities of a 100 mm long cross centered on the sampling point. Care was taken so that the four grab samples taken at each sampling point represented equal volumes. The samples were collected over the entire depth of the soil, which varied between 100 mm and 300 mm. Approximately 5.2 L of soil was collected for each sample. In addition, one composite sample was obtained for each cell by mixing equal parts of each of the six grab samples.

Figure 1. Illustration of Possible Field Sampling Point Location.



□ possible sampling point for sector 03

3.2.5 Sample Collection

The discrete samples were placed into plastic bags and mixed well. They were then separated as follows, except during the October sampling event when samples for T.E.H., soil moisture content and phenols were left in the plastic bags because of the high moisture content which could have created problems when trying to retrieve the samples from the glass jars:

a) for grab sample analysis at U of A:
   1) T.E.H. and soil moisture content: clear 500-mL glass jars were filled and Teflon liner and screw cap placed on;
   2) phenols: amber glass jars already containing 2 mL H_2SO_4 preservatives were filled, and Teflon liner and screw cap placed on;

b) to form composite sample equal volume of six grab samples were placed into new plastic bag and mixed well;

c) for composite sample analysis at U of A:
   1) T.E.H. and soil moisture content: as a) 1);
   2) Microtox® and microbial enumeration: volume placed in sterile plastic bag; and

d) for analysis at Enviro-Test required volume of composite sample was placed into soil bag provided.

3.2.6 Sample Handling

Sampling tools were decontaminated between sampling points at a location away from sample collection and separation to avoid cross-contamination using the following steps:

a) excess soil was scraped off the tools;

b) tools were washed with lab grade detergent (Extran 1000) and rinsed with distilled water;

c) tools were rinsed with isopropanol and rinsed again with distilled water; and

d) tools were allowed to air dry before the next use.

All liquid waste generated during equipment decontamination and sampling was collected in a container and then disposed in the runoff pond. All solid waste was disposed on site as directed by site operator.

All samples were packed in coolers containing ice packs and brought to U of A laboratory at the end of each day where they were stored appropriately. Samples being analyzed at Enviro-Test were dropped off on the last day of each sampling event.

3.2.7 Sampling Quality Control

During the May sampling event the following controls were prepared using commercial potting soil:

a) field blank control: stored in sterilized plastic bag;

b) field blank: sampled, placed in plastic bag and placed in collection jar;

c) trip blank: placed in collection jar at the lab, not opened and returned to lab in soil sample cooler; and

d) equipment blank: sampled and placed in collection jar.

These blanks were intended as sampling controls for T.E.H. analysis. Upon T.E.H. analysis of the field blank and field blank control they were found to be inappropriate as a control due to the high organic matter content which was extracted by the solvent. Furthermore the high within-sample variation in T.E.H. levels lead to the decision not to assess possible sampling contamination.

3.2.8 Safety

CSA approved hard hats (provided by IPL Inc.), work boots and Nomex (fire retardant) coveralls (provided by IPL Inc.) were worn at all times on site. In addition nitrile gloves and face shields were worn for decontamination activities.

3.2.9 Tracking and Reporting Activities

3.2.9.1 Sample Identification and Coding

The following alphanumeric sample coding system was used:

ED-b-nn-yymmdd

where:

-ED: two-letter code identifying Edmonton facility;

-b: letter identifying the cell from which soils samples will be collected (A,B,C, etc.). Not included for quality control samples;

-nn: two-character alphanumeric code identifying the sample type and station as follows:

01 to 06	grab samples within the cells;
07	composite sample prepared from grab samples 01 to 06;
FC	field blank control;
FB	field blank;
TB	trip blank;
EQ	equipment blank;

-yymmdd: six-character numeric code for the date on which the sample was collected (year/month/day).

3.2.9.2 Reporting

A Field Activities/Observations Report was produced for each sampling event. A Soil Sample Description sheet was used for visual description of each sample. Examples of these forms can be found at Appendix B.

3.3 Analytical Methods

3.3.1 General

The following storage and holding time requirements presented in Table 4 were adhered to at the U of A lab with a few exceptions for the microbial enumeration holding time.

Table 4. Sample Storage and Holding Times Requirements - Field Work 1995.

Analyte	Storage	Holding Time	Reference
T.E.H.	4°C	extract within 14 days, analyze within 40 days of extraction	US EPA, SW-846
Phenols	4°C, acidify with H_2SO_4	analyze within 28 days	APHA, 1994
Soil moisture content	4°C	preferably within 24 hours, but within 7 days	APHA, 1994
Microtox®	4°C, in dark	preferably within 2 weeks, but within 6 weeks	Environment Canada, 1991
Microbial enumeration	4°C	within 24 hours	APHA, 1994

Table 5 lists the analytical methods used for all samples and indicates where the analysis was performed as some of the soil analyses were performed at Enviro-Test Laboratories in Edmonton, AB.

Table 5. Analytical Methods - Field Work 1995.

Analyte	Analytical Method	Laboratory
T.E.H.	EPA SW846-3540, -8015 modified	U of A
Phenols	modified APHA 5530B, APHA 5530C	U of A
Metals Scan	EPA SW846-3051, -6010, APHA 3114C	Enviro-Test Laboratories (E.T.)
Mercury	EPA SW846, APHA 3112B	E.T.
TOC	CSSS 21.2	E.T.
SAR	CSSS 18.4	E.T.
% Saturation	CSSS 18.2.2	E.T.
pH	CSSS 16.3	E.T.
EC	CSSS 18.3.1	E.T.
Chloride	APHA 4110B-Cl	E.T.
Sulphate	APHA 4500F - SO4	E.T.
Ammonia	APHA 4500 - NH3	E.T.
Nitrate-N	APHA 4500 - NO3	E.T.
Orthophosphate	CSSS 8.4, APHA 4500 - P	E.T.
TKN	CSSS 22.2	E.T.
Soil moisture content	CSSS 51.2	U of A
Particle Size Distribution	CSSS 47.3	E.T.
Microtox®	Microtox® Manual, Vol. 2	U of A
Microbiological Enumeration (THB)	CSSS 27.3 mod, APHA 9215C	U of A

3.3.2 T.E.H.

3.3.2.1 Extraction

The sample was ground and sieved through a 2 mm sieve. A measured amount of the soil sample (approximately 10 g) was mixed with 10 g of anhydrous sodium sulphate in a cellulose extraction thimble. When the moisture content was too high for grinding and sieving the sample was mixed with a greater amount of sodium sulfate and mixed until the soil grains became loose. The thimble was placed into the Soxhlet extraction apparatus and spiked with100 µL of a 4.3 mg/mL 1-chlorooctane and 4.2 mg/mL 1-chlorooctadecane in methylene chloride solution to assess recovery. The sample was extracted for 16 hours with 150 mL methylene chloride. After the

extract was allowed to cool, it was concentrated using a rotary evaporator to a volume of approximately 2 mL; the extract was removed from the extraction flask and the flask was then rinsed to result in a final extract volume of approximately 5 mL. The final extract volume was quantified.

3.3.2.2 Quantification

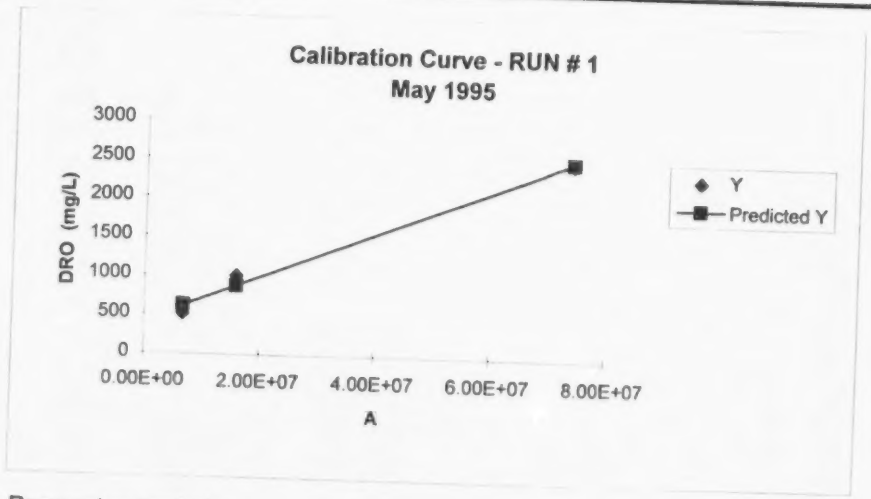
1 microlitre of the concentrated extract was injected onto a 30 m, 0.53 mm ID, 0.25 μm Rtx® -1 column and the resolved components were monitored using a flame ionization detector. A 5890A Hewlett Packard GC was used with a 7673A Automatic Injector. The carrier gas was helium and inlet pressure was set at 45 kPa resulting in a column velocity of 644 mm/sec. Injector and detector temperatures were set at 280°C and 310°C respectively and initial oven temperature at 40°C with a 12°C/min ramping of the temperature up to a final temperature of 310°C.

Restek® Diesel Fuel #2 Standard (unweathered) was used to quantify petroleum hydrocarbons between C_7 and C_{30}, referred to as Diesel Range Organics (DRO). 500, 1000 and 2500 mg/L standards were prepared and it was found that the response factors obtained (area/(mg/L)) were dependent on concentration. Regression analysis was used to obtain a concentration vs area response curve from which the sample concentrations could be calculated. Figure 2 illustrates a representative DRO calibration curve. Supelco® D2887 Quantitative Calibration Mix was used to determine the retention times associated with nC_7 and nC_{30} alkanes.

3.3.3 Phenols

The weighed soil sample, approximately 25 g, was mixed with 500 mL distilled water and the mixture was distilled in accordance with APHA 5530B. The second step involved pH-adjusting the distillate in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye was then extracted from the distillate with chloroform. HP 8452A Diode Array UV/VIS Spectrophotometer was used to read absorbance of samples and standards against a blank at 460 nm. Beer's Law fit was used to produce a calibration curve.

Figure 2. Typical DRO Calibration Curve.



Regression Analysis:

Conc = 424.517 + 2.8234E-5 * Area R^2 = 0.99

3.3.4 Metals Scan

Microwave nitric acid digestion was performed on soil samples and 16 metals (Ag, Ba, Be, Cd, Co, Cr, Cu, Mo, Ni, Pb, P, Sn, Sr, Tl, V, Zn) were analyzed by inductively coupled plasma (ICP) spectrophotometry in the digests. Mercury was analyzed by continuous cold vapour atomic absorption spectrometry on a digest with permanganate, nitric and sulfuric acids. Arsenic and selenium were analyzed by flameless atomic absorption spectrometry on a digest with sulfuric acid/persulphate and HCl. Routine metals (Ca, K, Mg, Na) were analyzed by ICP spectrophotometry on a filtrate of a saturated paste extract.

3.3.5 TOC

A wet oxidation-redox titration method was used to analyze organic carbon in the soil samples. This method was used as it is deemed suitable for comparative work on similar soils (CSSS, 1993).

3.3.6 EC

EC measurements were performed on the saturated paste extracts of the soil (CSSS, 1993).

3.3.7 Ammonia-N

Exchangeable NH_4^+ is defined as NH_4^+ that can be extracted at room temperature with a neutral K salt solution (CSSS, 1993). In this case available ammonia-N analysis was performed on a 1:2 extraction with 2 N KCl solution using an ion selective electrode method (APHA, 1994).

3.3.8 Nitrate-N

Automated colorimetry was used to determine available nitrate-N on a 1:2 extraction with 0.001 M $CaCl_2$ solution (APHA, 1994).

3.3.9 Orthophosphate - PO_4^{3-}

Automated colorimetry was used to determine available orthophosphate on a modified Kelowna extration of the soil samples (APHA, 1994). The extractant contained ammonium acetate and acetic acid.

3.3.10 Microtox®

Microtox® tests were performed on the WSF of soil samples. A 4:1 deionized water and soil mixture was shaken for 3 hours at 250 rpm, then allowed to settle for 2 hours. The decant liquid was then centrifuged for 15 minutes at 3000 rpm. The supernatant aqueous solution was collected for analysis (Chemex Inc., 1994; Symons and Sims, 1988).

3.3.11 Microbial Enumeration

THB were estimated on 1:100 soil : peptone water (1%) dilutions (CSSS, 1993). The spread plate method was used with a standard plate count agar (APHA, 1994). Plates were incubated for 7 days at 20°C.

3.3.12 Quality Control

The following laboratory quality control requirements were fulfilled.

3.3.12.1 T.E.H.

3.3.12.1.1 Precision

Method precision was estimated from the variance of triplicate analyses of six samples for each sampling event. Obviously this yields an overestimation of method precision as it also includes variability associated with contaminated soil. To minimize the effect of soil variability, variances calculated from triplicate analysis of composite samples were not considered. Coefficient of variation (COV) for triplicate analysis ranged between 5% and 50%, with a mean of 22%. There was no correlation between T.E.H. levels and COV. Sources of method variability include solvent extraction efficiency, extract recovery efficiency and instrument precision.

Duplicate or triplicate analysis of standards during each sampling event allowed for estimation of instrument precision. Variance was first evaluated on 1-chlorooctane and 1-chlorooctadecane i.e. single compounds. Within sampling event COV ranged between 0 and 20%, with a mean of 6%. For DRO standards analysis, within a sampling event COV ranged between 1% and 39%, with a mean of 10%. These results indicate considerable variation. Sources of variability include variations in column pressure head, column integrity and detector sensitivity.

Estimates of between-sampling event variability on DRO standards response factors yielded COV ranging from 13% to 27%, with a mean of 21% and with the higher concentration standard yielding the highest COV. Response factors from May analysis were not used in these calculations as the integrator used at that time yielded signal outputs of different orders of magnitude. Increasing or decreasing trends were not observed in the value of DRO response factors between May and October. All these potentially high sources of variation must be kept in mind when assessing the T.E.H. levels obtained in this study.

3.3.12.1.2 Recovery

High recoveries were consistently observed (106% to 150%). It became obvious that hydrocarbon compounds in the same elution range as the spiking compounds could produce these apparent high recoveries. Method blanks recoveries were used to get a more accurate estimate of normal recoveries. Sampling event mean recoveries were consistently greater than method blanks recoveries.

However both sample mean and method blank recoveries for October (38% to 74%) were substantially lower than those observed previously. Soils conditions could not justify the difference in recoveries as they were similar to those encountered in the previous sampling events. No clear evidence could be found as to the cause of these low recoveries. The fact that October DRO standards analyses yielded higher than mean response factors indicated that instrument sensitivity was not a problem. To compensate for these apparently low recoveries, DRO concentrations were corrected to 100% recovery.

3.3.12.1.3 Contamination

One glassware blank and one method blank were analyzed for each sampling event. For the glassware blank a thimble was placed in the Soxhlet extraction apparatus and for the method blank a thimble containing10 g sodium sulphate and one hundred microlitre of the 1-chlorooctane and 1-chlorooctadecane solution was placed in the Soxhlet extraction apparatus. The mean contamination associated with the method blanks was 5.9% of the DRO area where as contamination associated with the glassware blanks was not detected until the last sampling event where it was evaluated as 3.7% of the DRO area. Gas chromatography/mass spectrophotometry of blanks extracts identified the source of contamination as phthalates which are prevalent in a laboratory environment (syringe plungers, vial lids, etc).

3.3.12.2 Phenols

3.3.12.2.1 Accuracy

Because of difficulties with the analytical method which will be discussed later, duplicates of four water samples were sent for analysis at Enviro-Test Laboratories during the July sampling event. Results are included in Table 6. This comparison mainly illustrates the high variability of the results obtained in-house. It was therefore concluded that the analytical results obtained in-house should be considered as a maximum.

Table 6. Comparison of Phenols Analyses Results - July 1995.

Sample Name	U of A Result	U of A Replicate	Enviro-Test Replicate
ED-01-950725	1.44	0.00	
ED-02-950725	0.44		<1
ED-04-950725	9.47	0.00	<1
ED-05-950725	0.00	<1.00	<1
ED-07-950725	12.1		<1

(all results in µg/L)

3.3.12.2.2 Precision

Duplicates of some water samples were analyzed in-house in July 1995 in order to estimate method precision and results are shown in Table 6. Both samples yield COV's of 141%. This high variability was kept in mind when evaluating results. Overall instrument precision was estimated from triplicate analysis of each sample and standard. COV for sample analyses ranged from 0% to 173%, with a mean of 9% and the high COV being consistently associated with concentrations approaching 0. COV for standard analyses ranged from 1% to 11%, with a mean of 4%. Higher COV were associated with standard concentrations lower than 10 μg/L.

3.3.12.2.3 Contamination

Glassware blanks and method blanks were analyzed during the May sampling event. These analyses yielded phenols concentrations as high as 27 μg/L with high variability. Some of the corrective measures implemented are discussed in a subsequent portion of this report. Only method blanks were analyzed during the July and October sampling events. Results indicated contamination between 0 and 7 μg/L. These levels were considered to be insignificant.

3.3.12.3 Moisture Content

3.3.12.3.1 Precision

For each sampling event triplicates of at least six samples were analyzed in order to estimate method precision. This estimate also included sample variance. To minimize effects of sample variance COV from composite samples were not considered. COV ranged from 0.7% to 11% throughout the season with a mean of 3%.

3.3.12.4 Microtox®

3.3.12.4.1 Accuracy and Precision

Phenol standards were analyzed at the beginning and at the end of each batch of analyses. Results can be considered to be accurate when the phenol standard tests yield EC50 between 13 and 26 mg/L and in general, maintaining a COV of less than 20% is considered acceptable (Microbics Corporation, 1992).

Between May and October 1995, phenol standard tests yielded EC50 between 17 and 23 mg/L and COV for six phenol standard tests was 11%. The analytical technique was considered to be in control.

3.3.12.5 Microbiological Enumeration (THB)

Dilution of each sample was plated in triplicate. Geometric mean of the three counts was reported.

3.4 Results

3.4.1 Analytical Results

Detailed analytical results for T.E.H. and phenols can be found at Appendix C. All other analytical results are reported below.

3.4.2 Calculations - T.E.H. (C_7 - C_{30})

Explanation of methodology used to calculate T.E.H. (C_7 - C_{30}) concentrations and a sample calculation can be found at Appendix D.

3.4.3 Monitoring Results

For ease of comparison and discussion key results are tabulated below. Where relevant CCME interim criteria for commercial and industrial remediation are presented. As new soils were placed in Cells C and D in September, results pertaining to the new soils are indicated in parentheses to differentiate them from results pertaining to the original soils.

T.E.H. results for all sampling events are presented in Tables 7, 8, 9 and 10. T.E.H. results are per dry weight of soil and soil moisture content levels are included.

Table 7. Field T.E.H. and Soil Moisture Content Analyses Results- May 1995.

	Cell A		Cell C		Cell D	
	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)
-01	1.98E3	17	5.18E3	17	1.18E3	14
-02	2.16E3	14	1.88E3	15	3.35E3	14
-03	5.03E3	15	2.54E3	18	2.34E3	13
-04	2.36E3	17	1.17E3	13	6.94E2	13
-05	1.39E3	19	3.09E3	15	8.18E2	13
-06	3.04E2	11	1.15E3	14	3.74E2	10
-07	4.13E3	17	2.17E3	17	1.16E3	15
mean (-01 to -06)	2.20E3	16	2.50E3	15	1.46E3	13
st. dev.	1.57E3		1.52E3		1.15E3	
upper 90% confidence limit on the mean	3.26E3		3.52E3		2.23E3	

Table 8. Field T.E.H. and Soil Moisture Content Analyses Results- Jul 1995.

	Cell A		Cell C		Cell D	
	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)
-01	6.12E2	21	5.68E2	27	7.01E2	27
-02	6.17E3	23	1.25E3	24	4.26E2	19
-03	2.49E3	25	5.75E2	22	5.61E2	22
-04	9.16E2	22	1.29E3	22	7.74E2	22
-05	1.05E3	19	4.43E2	24	7.88E2	21
-06	1.68E3	27	1.12E3	23	8.37E2	26
-07	9.59E2	21	7.43E2	22	6.41E2	22
mean (-01 to -06)	2.15E3	23	8.74E2	23	6.81E2	23
st. dev.	2.08E3		3.86E2		1.58E2	
upper 90% confidence limit	3.55E3		1.13E3		7.87E2	

Table 9. Field T.E.H. and Soil Moisture Content Analyses Results- Sep 1995.

	Cell C		Cell D	
	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)
-01	9.85E2	24	9.46E2	15
-02	9.72E2	25	8.89E2	17
-03	1.25E3	10	1.72E3	19
-04	2.92E2	17	1.83E3	18
-05	1.09E4	13	1.35E3	17
-06	4.70E2	24	1.53E2	19
-07	9.52E3	18	1.03E3	17
mean (-01 to -06)	2.48E3	19	1.15E3	18
st. dev.	4.14E3	6	6.21E2	2
upper 90% confidence limit	5.26E3		1.57E3	

Table 10. Field T.E.H. and Soil Moisture Content Analyses Results - Oct 1995.

	Cell A		Cell C		Cell D	
	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)	T.E.H. (mg/kg)	moisture (%)
-01	2.99E2	22	3.44E2	28	6.36E2	16
-02	3.67E2	22	5.50E2	25	5.09E2	15
-03	4.49E2	23	5.97E2	14	3.27E2	27
-04	8.38E2	26	8.03E2	15	9.19E2	18
-05	1.02E3	27	3.26E3	14	5.63E2	24
-06	6.31E2	29	1.18E3	30	4.49E2	24
-07	4.79E2	23	8.20E2	20	3.92E2	19
mean (-01 to -06)	6.01E2	25	1.12E3	21	5.67E2	21
st. dev.	2.83E2		1.08E3		2.02E2	
upper 90% confidence limit	7.91E2		1.85E3		7.03E2	

Mean phenol levels are presented in Table 11.

Table 11. 1995 Field Mean Phenols Levels.

(all results in μg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 95	180	604	706
July 95	107	25	85
October 95	309	(410)	(455)

( ) indicate new soils

Results from metal analyses performed on composite samples from each cell are included in Table 12 and 13.

Table 12. Field Metals Analyses Results - May 1995.

(all results in mg/kg)

Metal	CCME criteria (mg/kg)	Cell A	Cell C	Cell D
Arsenic	50	5.2	4.4	4.8
Barium	2000	175	177	173
Beryllium	8	<1	<1	<1
Cadmium	20	<0.5	<0.5	<0.5
Cobalt	300	7	7	7
Chromium	800	23.1	26.0	20.0
Copper	500	22	23	23
Mercury	10	0.02	0.02	0.02
Molybdenum	40	<1	<1	<1
Nickel	500	<2	26	24
Lead	1000	11	12	10
Tin	n/a	<5	<5	<5
Selenium	10	0.3	0.3	0.2
Silver	40	<1	<1	<1
Strontium	n/a	44	43	42
Thallium	n/a	<1	<1	<1
Vanadium	n/a	27	28	25
Zinc	1500	66.8	76.5	68.2

Table 13. Field Metals Analyses Results - Oct 1995.

(all results in mg/kg)

Metal	CCME criteria (mg/kg)	Cell C	Cell D
Arsenic	50	2.7	2.2
Barium	2000	230	230
Beryllium	8	< 1	< 1
Cadmium	20	< 0.5	< 0.5
Cobalt	300	8	7
Chromium	800	57.9	43.7
Copper	500	28	24
Mercury	10	0.04	0.04
Molybdenum	40	< 1	< 1
Nickel	500	65	40
Lead	1000	29	17
Tin	n/a	7	6
Selenium	10	0.5	0.3
Silver	40	< 1	< 1
Strontium	n/a	60	57
Thallium	n/a	< 1	< 1
Vanadium	n/a	36	29
Zinc	1500	160	90.9

Ammonium-N, nitrate-N and orthophosphate analyses results are presented in Tables 14, 15 and 16 respectively.

Table 14. 1995 Field Ammonium-N Analyses Results.

(all results in mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 95	2.6	1.6	6.8
July 95	98	182	95
Sep 95	N/A	(0.6)	(0.8)
Oct 95	38.8	(39.6)	(18.6)

( ) indicate new soils

Table 15. 1995 Field Nitrate-N Analyses Results.

(all results in mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 95	9.0	5.4	13.0
July 95	172	252	280
Sep 95		(34.2)	(30.6)
Oct 95	240	(94.0)	(62.0)

( ) indicate new soils

Table 16. 1995 Field Orthophosphate-P Analyses Results.

(all results in mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 95	5.9	13.2	12.1
July 95	28.0	37.5	75.0
Sep 95		(3.1)	(2.4)
Oct 95	38.0	(9.3)	(5.8)

( ) indicate new soils

In order to assess any differences in nutrient status between cells, molar C:N:P ratios were calculated based on the mean T.E.H. levels at the time of sampling and assuming 100 % carbon content. Although assuming carbon content of petroleum hydrocarbons is a poor assumption, it was used in order to retain comparability between 1994 and 1995 results. Sample calculation can be found at Appendix E. The results are presented in Table 17.

Table 17. 1995 Field Molar C:N:P Ratios.

Sampling Event	Cell A	Cell C	Cell D
May 95	100 : 0.5 : 0.1	100 : 0.2 : 0.2	100 : 1.0 : 0.3
July 95	100 : 11 : 0.5	100 : 43 : 1.7	100 : 47 : 4.3
Sep 95		(100 : 1.2 : 0.04)	(100 : 2.3 : 0.08)
Oct 95	100 : 40 : 2.4	(100 : 10 : 0.3)	(100 : 12 : 0.4)

( ) indicate new soils

Particle size analyses results are presented in Table 18.

Table 18. 1995 Field Particle Size Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 95	37.6% sand	36.6% sand	35.6% sand
	21.0 % silt	20.0 % silt	22.0 % silt
	42.4 % clay	44.4 % clay	40.4 % clay
Oct 95		(42.9 % sand)	(44.1 % sand)
		(21.3 % silt)	(20.0 % silt)
		(35.9 % clay)	(35.9 % clay)

( ) indicate new soils

TOC results are presented in Table 19.

Table 19. 1995 Field TOC Analyses Results.

(%)

Sampling Event	Cell A	Cell C	Cell D
May 95	2.3	2.1	2.3
July 95	3.3	2.7	3.6
Oct 95	2.7	(2.2)	(1.8)

( ) indicate new soils

pH, SAR and EC results are presented in Tables 20, 21 and 22 respectively.

Table 20. 1995 Field pH Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 95	7.3	7.4	7.4
July 95	7.2	7.3	7.2
Oct 95	7.8	(7.8)	(7.7)

(CCME criteria: 6 - 8)

( ) indicate new soils

Table 21. 1995 Field SAR Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 95	0.7	1.0	0.4
Jul 95	0.9	0.6	0.5
Oct 95	1.0	(0.8)	(0.7)

(CCME criteria: <12)
( ) indicate new soils

Table 22. 1995 Field EC Analyses Results.

(µS/cm)

Sampling Event	Cell A	Cell C	Cell D
May 95	3900	1200	2600
July 95	2900	3600	4300
Oct 95	3950	(1870)	(1720)

(CCME criteria: <4000 µS/cm)
( ) indicate new soils

Microtox® analyses results are presented in Table 23.

Table 23. 1995 Field Microtox® Analyses Results.

(EC50, 5 min)

(all results are as % concentration of undiluted WSF)

Sampling Event	Cell A	Cell C	Cell D
May 95	>100	>100	>100
July 95	>100	>100	>100
Oct 95	> 100	(29 - > 100)	(> 100)

( ) indicate new soils

THB counts are reported in Table 24. For each sample the mean and standard error of three plate counts are reported.

Table 24. 1995 Field THB Counts Results.

(Colony Forming Units (CFU)/g)

Sampling Event	Cell A	Cell C	Cell D
May 95	1.12E7 ± 0.06E7	7.67E6 ± 0.16E6	6.06E6 ± 0.10E6
July 95	1.30E7 ± 0.02E7	8.72E6 ± 0.32E6	8.57E6 ± 1.23E6
Oct 95	8.60E5 ± 0.12E5	(6.65E4 ± 0.34E4)	(6.60E5 ± 0.49E5)

( ) indicate new soils

3.5 Discussion

3.5.1 Monitoring Results

3.5.1.1 T.E.H.

3.5.1.1.1 Analytical Method

Some of the difficulties associated with the analytical method used will be discussed here. Selection of a proper standard was difficult. Ideally if a sample of the actual contaminants are available, it should be used as the standard. As all contaminated soils are stockpiled in one location at IPL Edmonton transfer station, many types of hydrocarbon products can be found as contaminants and it was apparent during the project that some of the soil samples were contaminated with more than one petroleum product. Unfortunately, a standard analytical method for petroleum-contaminated soils has not been specified by the regulatory agency, in this case being NEB, or by other regulatory bodies such as CCME (CCME,1993) and therefore appropriate standards have not as yet been specified. Because of time and financial considerations, Restek Diesel Fuel #2 standard was identified at the

beginning of the project as being satisfactory and suitability of other standards was not assessed.

Although these were not quantified in this study, other possible sources of errors arise from the extraction step. The solvent extract contains not only fossil hydrocarbons but also lipids, waxes and hydrocarbons of nonfossil origin; in mineral soils 1 to 5% of the organic matter may be extracted by organic solvents (Bossert and Bartha, 1984). Biogenic hydrocarbons have been identified in soil at levels of up to 50 ppm (Chaîneau et al., 1995) and that level did not change over the course of a 300-day biodegradation study indicating that part of the biogenic hydrocarbons are protected from biodegradation, most likely because they are bond to the organomineral matrix of the soil in a way that prevents accessibility for microorganisms. Other studies have demonstrated that in the upper layer, where biodegradation is effective, biogenic alkanes are degraded together with fossil hydrocarbons (Oudôt et al., 1989); the terrestrial biogenic production of aromatic hydrocarbons is thought to be insignificant. As well soil microorganisms and macroorganisms exude various organic chemicals as a part of their metabolic functions including alkanes, alkanoic acids, alkanols, alkanoids, cyclic alkanes, methyl alkanes, organic cyanides and numerous aromatic derivatives. Although the remediation goal in this study (2000 mg/kg) did not warrant such procedures, additional pretreatment steps such as passage of the sample through a silica gel column can be used to allow only petroleum-based hydrocarbons to be quantified (Troy et al., 1994). The extracted material also includes hydrocarbon biodegradation intermediates such as solvent-soluble long-chain fatty acids (Bossert et al, 1984).

3.5.1.1.2 Monitoring Results

As results presented in Table 7 to 10 indicate all soils were successfully remediated throughout the 1995 season. As mentioned in section 1.2.1, the soils in a cell are considered to be remediated when the 90% confidence limit on the mean of six samples is equal to or less than 2000 mg/kg T.E.H. There were decreases in mean T.E.H. levels of 65% in Cell C and 59% in Cell D between May and July 1995. Cell A however did not experience a comparable reduction in T.E.H. levels during that

same period. October results indicated a 72% decrease in T.E.H. levels for Cell A between July and October 1995. May 1995 results for all cells indicated that hydrocarbons below nC$_{12}$ were absent therefore minimizing the amount of hydrocarbons that could have been subject to volatilization. Biodegradation was thought to be occurring in Cells C and D as July fingerprints still contained hydrocarbons in the same range (nC$_{12}$ to nC$_{40}$) indicating that lower molecular weight hydrocarbons were not preferentially removed. Some of Cell A October fingerprints also showed that hydrocarbons over the whole range had been removed.

New soils were placed in Cells C and D after the July sampling event and September results indicated that Cell D was already below the remediation limit. The soil was kept in place so that full characterization could be carried out during the October sampling event. October results indicated decreases of 55% for Cell C and 51% for Cell D between 13 Sep 1995 and 17 Oct 1995. Whereas most samples contained hydrocarbons in the > nC$_{14}$ range in Cell C, Cell D samples also contained hydrocarbons in the nC$_8$ to nC$_{14}$ range. Obviously some hydrocarbons could have been removed through volatilization but some fingerprints also showed that *n*-alkanes over the whole range were being removed.

Although no rigorous analysis was made to identify the specific contaminant type, the presence of a wide range of hydrocarbon compounds including very high molecular weight compounds indicated possible crude oil contamination. Because of differences in analytical methods, contaminant levels and operational conditions, comparison between studies are difficult. The results obtained here are comparable to those of Ju et al. (1993) which involved similar conditions and where a 75% reduction in Total Petroleum Hydrocarbons occurred in a 40 day period, refer to Table 2.

Rate constants in each cell were evaluated based on first-order kinetics and results are presented in Table 25. Again comparisons with other studies are made cautiously. These rates are within the range or somewhat higher than rate constants from 0.04 to 0.08 week^{-1} reported by Carberry (1994) for full-scale bioremediation of medium molecular weight petroleum contamination.

Table 25. 1995 Field Bioremediation Rate Constants.

	First-Order Rate Constant ($week^{-1}$)
Cell A (May - Oct)	0.07
Cell C (May - July)	0.12
Cell D (May - July)	0.10
Cell C (Sep - Oct)	0.16
Cell D (Sep - Oct)	0.14

Combined decreases in the variability and value of hydrocarbon concentrations have been reported in bioremediation systems (McNicoll and Baweja, 1995). As reductions in standard deviations between sampling events demonstrate, refer to Tables 7 to 10, this effect was observed in this study. This homogenization is believed to be a combination of mixing effect (from tilling of the soil) and bioremediation.

3.5.1.2 Phenols

3.5.1.2.1 Analytical Method

Because the calibration utilizes phenol as a standard, the analytical method yields values which represent the minimum concentration of phenolic compounds. In addition, it does not determine certain para-substituted compounds (APHA, 1994).

There were many problems identified with the analytical procedure used for soil phenols analysis. In May the older solvent used in the extraction step for some of the samples was thought to be partly responsible for some of the high results obtained. At that time analyses of glassware blanks and method blanks also revealed possible contamination problems.

A few steps were added to the analytical procedure to avoid suspected contamination found in the first sampling event. All the glassware used was rinsed with iso-propanol or chloroform, depending on its intended use, after being washed.

3.5.1.2.2 Results

Not considering May 1995 results, there was a 53 % reduction in the amount of phenols in Cell A between October 1994 and July 1995; the levels of phenols in Cells C and D did not experience important changes between October 1994 and July 1995. These two cells had experienced considerable reductions, in the order of 80%, between July 1994 and October 1994. October results indicated an increase in mean phenol levels in Cell A since July 1995 although the standard error was quite high. CCME criteria do not specify remediation guidelines for total phenolic compounds. They do specify a limit of 10 000 μg/kg for each nonchlorinated phenol and 5000 μg/kg for each chlorophenols.

Because of the difficulties encountered in the analysis of soil phenolic compounds the significance of the changes in phenols concentrations will not be discussed in detail. It can only be stated that decreases are possible as phenolic compounds have been shown to be biodegraded (Vipulanandan et al., 1994, Evangelista et al., 1990). Phenols have also been identified as biodegradation by-products of aromatic hydrocarbons (Wilson and Jones, 1993) which could possibly accumulate and cause increases in phenol levels.

The analytical method used for soil phenols analysis is a method intended for water analysis and is subject to interference by oils which were present in the soil being analyzed. CCME has recommended EPA method 8270B for phenolic compounds analysis as the only generally applicable method that is recommended for use with soils and sediments (CCME, 1993).

3.5.1.3 Metals

All metals analyses results were well below CCME criteria for commercial and industrial remediation. As far as possible effects on the bioremediation process, heavy metals such as lead, mercury, cadmium, chromium and nickel are of concern. Concentrations associated with toxic effects have not been identified for many of these metals. As can be seen in Tables 12 and 13 results obtained in this study support the findings of Frankenberger (1992) that lead concentrations of up to 1000 ppm and cadmium concentrations of up to 100 ppm did not interfere with the

mineralization of a petroleum product. When assessing possible toxic concentrations, metals speciation should also be considered as it will affect its bioavailability and toxicity. Unfortunately ICP analysis does not yield information on metal species and oxidation state.

3.5.1.4 Available Nutrients

Results included in Table 17 indicate that when compared within sampling events nitrogen levels are constantly in the same ranges i.e. either well below or well above the target ratio of 100 : 5. Orthophosphate levels were similar in May 1995 but July 1995 results show a deficiency in phosphorous in Cell A but excesses in Cells C and D. Differences in nutrient status between Cell A and Cells C and D will be discussed further in the following sections.

The nutrient status of the new soil in Cells C and D between September 1995 and October 1995 will be discussed here. Review of the facility log book revealed that nutrients were not added between the Sep and Oct sampling event until five days prior to the Oct sampling event. The fact that the C : N : P ratio was 100 : 1 : 0.05 and 100 : 2.3 : 0.08 for Cells C and D respectively in September and that these ratios can be assumed to have prevailed for most of the 1-month period between sampling events does not seem to have affected the removal of hydrocarbons. As discussed in section 3.5.1.1.2 volatilization could have been part of the hydrocarbon removal process as the soil was recently placed in the cells. As well the soil could have had important amounts of organic nutrients being utilized by the microorganisms. Organic nitrogen levels are not accounted for in the available nitrogen analysis performed on the soil samples.

3.5.1.5 Soil Texture

The high clay content of the soils did not seem to affect the biodegradation process. As presented in Table 18 clay content varied from 35.9% to 44.4%. Although there were some concerns over the high clay contents with respect to hydrocarbon bioavailability (CH2M Hill, 1994), information provided in the literature review indicates that binding would be of concern mainly for biodegradation

intermediates. The low T.E.H. levels combined with Microtox® test results, to be discussed below, eliminate concerns over the possible presence of clay-bound biodegradation intermediates.

It has also been stated that tillage operations may be adversely affected by clay content in excess of 40% (McGill, 1978). It is thought that this would be true in cases of high moisture content, as was seen in 1994, see Appendix F for a detailed discussion. However, as moisture content was controlled early in the season in 1995, the soil could be effectively tilled.

3.5.1.6 TOC

TOC levels were similar in all soils and did not seem to affect the bioremediation process. As organic matter content is usually considered to be 1.724 to 2.000 times the organic carbon content, these results are within the range for typical farmland topsoils which may contain from 2 to 10 % organic matter (Pierzynski et al., 1994).

3.5.1.7 Routine Soil Chemistry

Routine soil chemistry results indicated suitable conditions for bioremediation.

As indicated in Table 20, all soil pH results were within the optimal 7 to 8 pH range for biodegradation by a mixed bacterial-fungal community (Bossert and Bartha, 1984).

The SAR is a useful index of the sodicity or relative sodium status of aqueous soil extracts. It is calculated as:

$$SAR = [Na^+]/[Ca^{2+} + Mg^{2+}]^{0.5} \quad (2)$$

where concentrations are in $mmol \cdot L^{-1}$ (CSSS, 1993). As Na^+ ions are highly hydrated, loosely held monovalent ions, an overabundance can cause dispersion which in turn will lead to lower permeability and swelling. As Table 21 results indicate, the CCME upper limit for commercial and industrial remediation of 12 was never exceeded (CCME, 1991).

The EC of a soil determines the total solute concentration in a soil extract. It reflects the content of soluble salts in a soil which can have detrimental effects on biological activity. Although conductivity levels are usually assessed against crop growth it can also have adverse effects on soil structure. As detailed in Table 22, EC results from the 1995 sampling are indicative of soils where yields of very sensitive crops would be restricted (CSSS, 1993). The only CCME criteria for commercial/industrial land use which was exceeded was the EC in soils from Cell D in July 1995, see Table 22.

3.5.1.8 Microtox®

3.5.1.8.1 Analytical Method

It has been reported that the Microtox test shows good correlation with traditional invertebrate and vertebrate toxicity tests (Wang and Bartha, 1994). The system makes use of a bioassay in which bioluminescent bacteria (*Photobacterium phosphoreum*) produce light as a result of a complex set of energy-producing reactions. Inhibition in any one of a multiple number of enzymes involved in this process causes a change in the rate of light emission. As mentioned in section 3.3.10 the test procedure was applied to the WSF of soil extracts. The relative toxicity of the WSF is of interest because chemical compounds that can be extracted with water represent the potentially leachable fraction of a waste or any intermediate chemical detoxification products. In fact the WSF poses the greatest threat to groundwater contamination (Dasappa and Loehr, 1991).

3.5.1.8.2 Results

The soil in all three cells were assessed as nontoxic (EC50>100%) during the first two sampling events and during the last sampling event in Cell A, see Table 23. The July results indicated that although the T.E.H. levels were considerably higher in Cell A, Table 8, the toxicity levels of the WSF were similar in all three cells. The hydrocarbons remaining in Cell A at the concentration found were either simply not toxic or not water soluble and therefore not likely to leach out of the soil.

In October, tests performed on the WSF from the new soils from Cells C and D indicated the samples to be non-toxic i.e. all assays yielded EC50's above 100% except for one extract from Cell C which was assessed as very toxic. This would support Cell C T.E.H. results for the new soil, Tables 9 and 10, which indicated the presence of contamination hot spots.

It is interesting to note that the higher toxicity observed in the new soil from Cell C was associated with lower T.E.H. concentrations, Table 10, compared to May T.E.H. levels for all three cells, Table 7, for which there was no toxicity detected. This is an indication of the presence of more soluble components in Cell C. This could also be due to differences in soil conditions, such as the lower clay content in the new soil from Cell C. Nevertheless, this observation shows that numerical T.E.H. level remediation goals do not guarantee toxicity-free WSF.

3.5.1.9 Microbial Enumeration

May and July 1995 results for all cells, Table 24, are within the normal range for fertile soils, that is from 10^5 to 10^8 CFU/g (Alexander, 1977). However it is known that the standard plate count agar yields lower counts than other agars used in the plate count technique (APHA, 1992). Cell A experienced a decrease in THB from the 10^7 CFU/g range in May and July 1995 to the 10^5 CFU/g range in October 1995. Levels in the 10^4 CFU/g range in the new soils from Cells C and D were also lower than typical levels found during the May and July monitoring events. These lower counts could be the result of the lower soil temperature at the time of sampling (about 10 °C) or may indicate the diminution or absence of utilizable substrate at that time.

Changes in microbial numbers are often observed throughout the biodegradation process. Levels as high as 10^{11} bacteria/g soil for THB and 10^{10} bacteria/g soil for HUB have been reported (Chaîneau et al., 1995). These changes have been shown to occur within weeks of the hydrocarbon contamination event with levels returning to normal within six to eight weeks (Wang and Bartha, 1994). However, such changes were not observed within the 1995 monitoring framework.

3.5.2 Cell A vs Cells C and D T.E.H. Results

3.5.2.0 General

It was suspected that the high T.E.H. levels in Cell A in May and July were due to hot spots, that is highly contaminated areas with T.E.H. levels greater than 2000 mg/kg. However whereas May results indicated the presence of such hot spots in both Cells C and D, July results pointed to their disappearance. Possible differences in treatment and/or contamination types that could have slowed down the biodegradation process in Cell A were investigated. A detailed analysis of 1994 and 1995 soil and treatment conditions is included at Appendix F. Only consequential results will be discussed here.

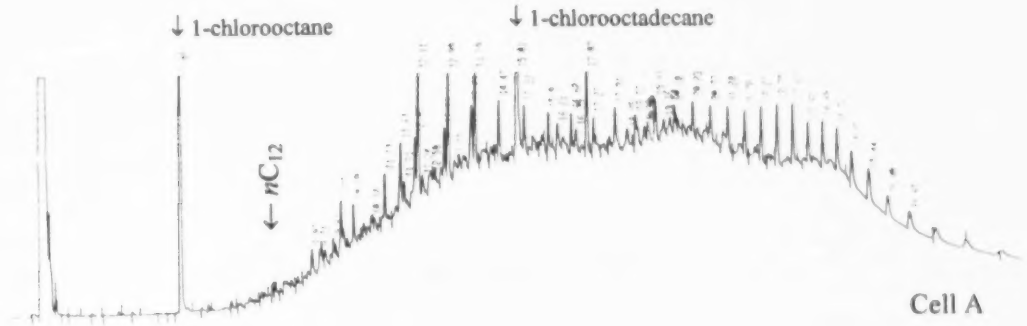
3.5.2.1 Soil Conditions

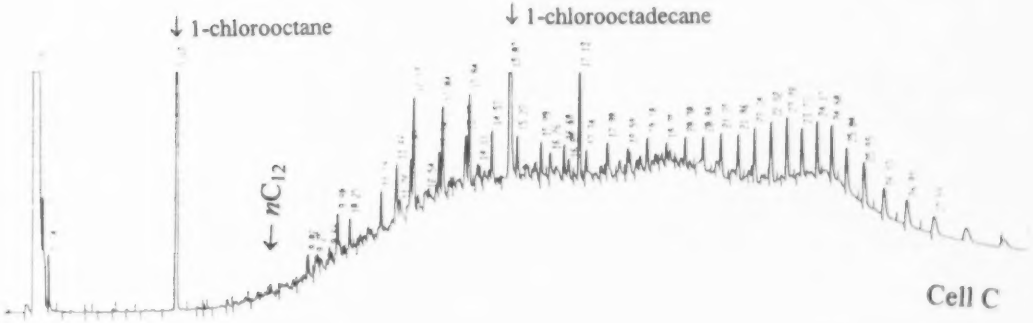
Lower zirconium and nickel levels were observed in Cell A, Table F1. Neither of these two metals have been identified as essential nutrients for most microorganisms (Alexander, 1977). The absence of Ni in Cell A in May 1995 (method detection limit (MDL): 2 mg/kg) was considered to be an effect of soil heterogeneity as it was present in May 1994 in similar concentration as Cells C and D. Nutrient conditions in May 1995, Tables F9 and F12, were similar in all cells and similar amounts of inorganic nutrients were added in June 1995, Table F18. Considering the fact that T.E.H. levels were similar in all cells in May 1995, Table 7, availability of nutrient was not thought to have caused the retardation in Cell A. A significantly higher microbial count (t-test, $\alpha = 0.05$) was observed in Cell A in May 1995, Table F16, which would not cause interference with the biodegradation process and it was thought that, if any, the effect would be to promote it.

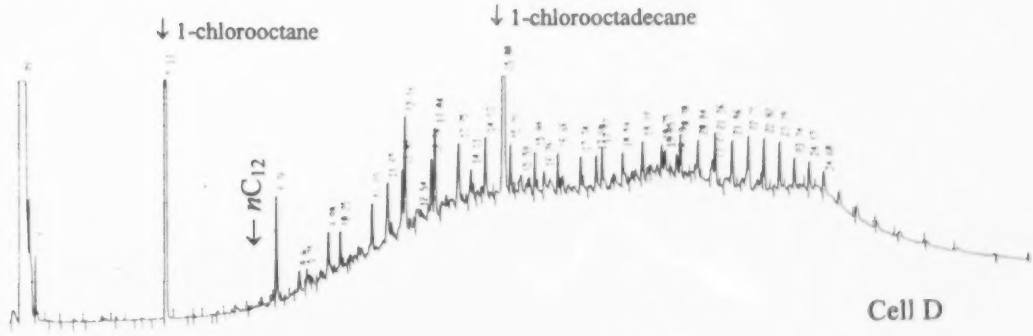
3.5.2.2 Chromatographic Fingerprints

May 1995 fingerprints indicated that all cells contained hydrocarbons in the $>nC_{12}$ to nC_{40} range. Typical chromatographic fingerprints from T.E.H. analysis for samples from Cells A, C and D are included in Figure 3. All samples were qualitatively compared on the basis of their resolved areas to unresolved area ratios.

Figure 3. Typical Chromatographic Fingerprints from T.E.H. Analysis for Cells A, C and D - May 1995.







The resolved hydrocarbons are alkanes that appear as specific peaks on the gas chromatograph fingerprints (Troy et al., 1994). As discussed already alkanes are considered the most readily degraded component of a hydrocarbon mixture so that major differences in the resolved to unresolved area ratios could be an indication of differing biotreatability potentials. Major differences were not apparent. It is acknowledged that this assessment was very qualitative, however October 1995 results, which indicated that Cell A soils were eventually subject to a comparable reduction in T.E.H. levels, did not warrant a more detailed investigation.

3.5.2.3 Summary

Soil conditions, treatment operations or chromatographic fingerprint analyses did not indicate differences in Cell A that could have caused the apparent retardation in biodegradation. As reduction in T.E.H. levels in Cell A between July and October 1995 was similar to those observed in Cells C and D between May and July there is a possibility that July results were not caused by the absence of biodegradation but were simply an indication of contaminated soil heterogeneity. This highlights the importance of tilling in the bioremediation process as it contributes to reducing contaminated soil heterogeneity. For monitoring pruposes, increasing the number of samples taken from each cell would also contribute to reducing the magnitude of the confidence limits on the mean T.E.H. levels therefore minimizing the effects of contaminant heterogeneity.

3.5.3 1994 vs 1995 Results

3.5.3.0 General

As the bioremediation process was successful in 1995, differences in soil conditions and treatment operations between 1994 and 1995 were reviewed. A detailed analysis of 1994 and 1995 soil and treatment conditions is included at Appendix F. Only consequential results will be discussed here.

3.5.3.1 Soil Conditions

Differences in analytical results for metals, Table F1, and EC, Table F2, between 1994 and 1995 were attributed to variations in analytical methods, specifically extraction methods. Conditions with respect to these two parameters were considered to be suitable for bioremediation. SAR, Table F4, and pH results, Table F6, throughout 1994 and 1995 were indicative of suitable conditions for bioremediation. Higher moisture contents were observed in all cells in June 1994, Table F13, which could have created oxygen deficiencies.

As assessed by molar C:N and C:P ratios there appeared to be nutrient deficiencies in all cells in 1994, Tables F9 and F12 respectively. The results from 1995 show that proper C:N and C:P ratios were attained between May and July 1995, Tables F9 and F12 respectively. Although there was a decrease in mean T.E.H. levels in Cells C and D between that same period, Tables 7 and 8, the situation in Cells C and D between September and October 1995, where decreases in mean T.E.H. levels of 55% and 51% in Cells C and D respectively occurred even though the target C:N:P ratios were not attained, preclude from drawing the conclusion that proper C:N:P ratios were essential to the success of the bioremediation process in 1995.

Microbial enumeration results for 1994 and 1995 were not readily comparable, as HUB were enumerated in 1994 as reported in Table F 16. May and July 1995 results for all cells were within a normal range for soils, that is from 10^5 to 10^8 CFU/g (Alexander, 1977).

3.5.3.2 Treatment Operations

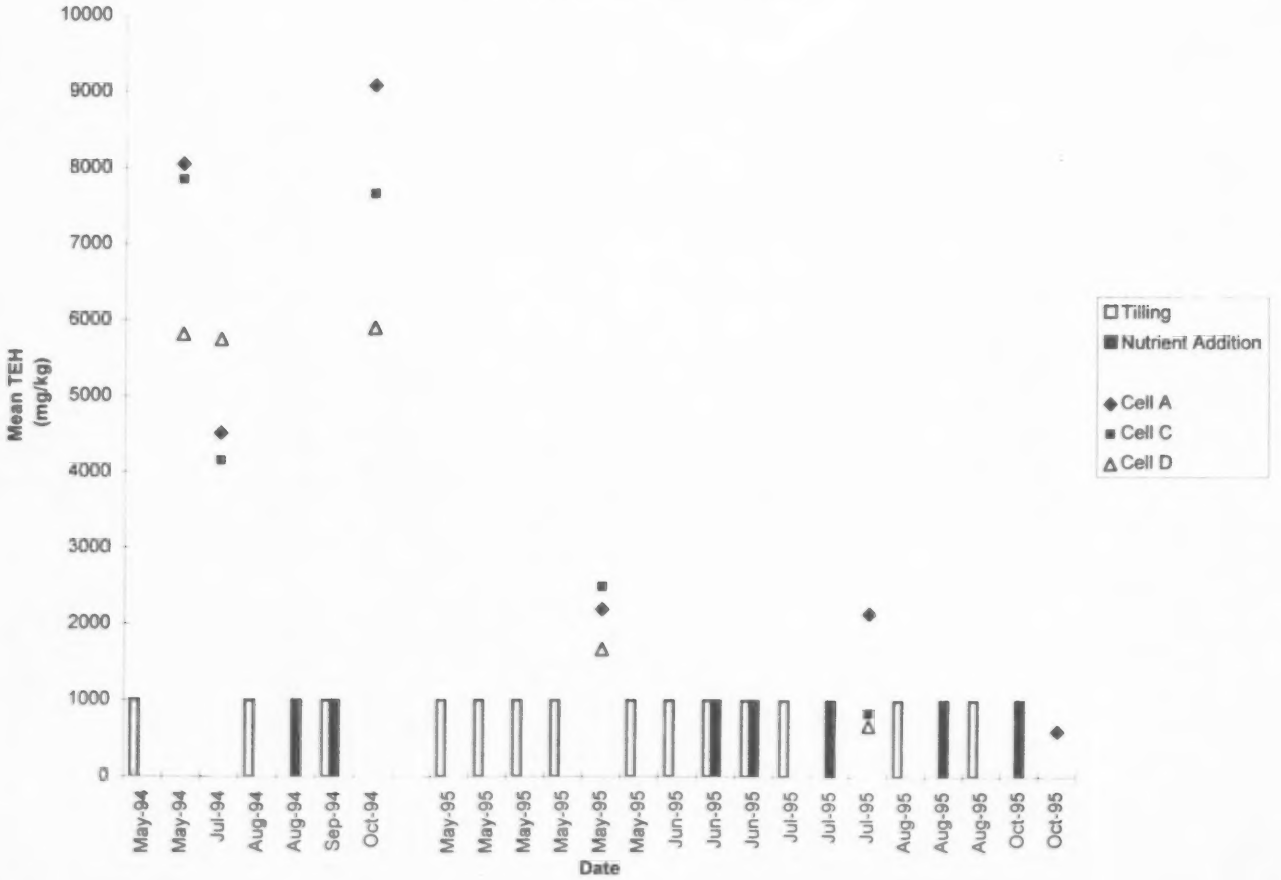
A major difference between 1994 and 1995 was the tilling frequency. Whereas the cells were only tilled three times in 1994, they were tilled almost weekly throughout May and June 1995. Although the frequency decreased thereafter, the soil was tilled three times in the next two months. The difference in tilling and nutrient addition frequency between 1994 and 1995 and the effect on mean T.E.H. concentrations in the cells is illustrated in Figure 4; it should be noted that tilling and nutrient addition events apply to all three cells except for August and October 1995 where treatments pertain to Cell A only. The importance of tilling was discussed in

sections 2.3.6.2, 2.3.6.4 and 2.3.6.7 with respect to moisture content control, oxygen availability and enhancement of clay penetration. Through discussions with IPL personnel, it was noted that the aggregate sizes observed in 1995 represented a major improvement from 1994 conditions, where much larger clumps of soils were present. Although quantitative assessment of clump sizes was not done in 1994, it was noted in 1995 that the soils contained aggregates of up to 7 cm in diameter. It was believed that the increase tilling frequency was instrumental in producing the smaller aggregate sizes.

Tilling action is also believed to redistribute the oil, nutrients and microorganisms, creating new points of attack for the microorganisms (Harmsen, 1991). As was discussed in section 2.5.2.3, soil homogeneity is desired as it alleviates difficulties associated with the interpretation of highly variable data caused by what as been referred to in this study as contamination hot spots.

It was noted in the facility logbook that wet soil conditions prevailed throughout June and July 1994 due to weather conditions and drainage problems within the cells, and as a result, access to the cells was restricted (CH2MHill, 1994). While weather conditions cannot be controlled, drainage problems within the cells are controllable. The high clay content of the soils at that time most likely compounded any drainage problems. As all types of soils must be treated efficiently it is essential that drainage performance be optimal. Design specifications for the construction of the cells (Hardy BBT Limited, 1991) should be reviewed to ensure they are still met.

Figure 4. Summary of 1994 and 1995 Field Treatments and Mean Soil TEH Concentrations



4.0 BENCH-SCALE OPTIMIZATION EXPERIMENT

4.1 Rationale

In 1995 field results identified two factors that could have contributed to the success of the bioremediation process. They were inorganic nutrient supplements to the soils, and frequent soil tilling contributing to moisture control and reduced clod sizes leading to improved oxygen availability.

Through visual observation of the soil samples during the 1995 remediation season, it was noted that the soils contained aggregates of up to 7 cm in diameter and some aggregates were very hard to separate by hand. Ju et al. (1993) investigated the effects of pulverization on soil bioremediation; the pulverization equipment they used reduced soil clods of up to 5 cm in diameter down to less than 0.5 cm in diameter. In laboratory experiments, soil respiration rates increased 3.44 times after the soil was pulverized; field tests showed about 25% reduction in treatment time when a pulverizer was used to aerate and grind the soils instead of disc plowing. Such soil processing was considered as a possibility to optimize the bioremediation process at IPL.

The varied effects of inorganic nutrient addition were discussed in section 2.3.6.5 with respect to contamination type and soil conditions. This study was undertaken to qualify and, if significant, quantify the effects of inorganic nutrients addition on T.E.H. removal from contaminated soils recovered from IPL stockpile. This was done under controlled conditions, similar to those provided in the field.

4.2 Aim

The aim of this experiment is to compare and contrast the effects of 1) reduction in size of soil aggregates and 2) adjustment of soil available nutrient levels to a C:N:P of 100:5:1 on the removal of hydrocarbons from contaminated soils.

4.3 Experimental Methods

4.3.1 Soil Preparation

Approximately 0.068 m^3 of soil was collected from IPL contaminated soils stockpile at the Edmonton transfer station. The soil was then mixed in a cement mixer for approximately 15 min in an effort to decrease heterogeneity.

4.3.2 Initial Soil Characterization

4.3.2.1 Aggregate Size Distribution

Aggregate size distribution was determined from three samples, approximately 1 kg in weight, in the following manner: (1) weigh sample, (2) separate the aggregates on the basis of the following size classes: 5 - 10 cm, 2 - 5 cm, 1 - 2 cm and < 1 cm and (3) weigh soils in each size class. Results were expressed as percentage weight of each sub-sample compared to sample weight. Aggregate sizes were determined on the basis of their longest length. Aggregate size distributions are reported in Table 26.

Table 26. Bench-Scale Soil Aggregate Size Distributions Analyses Results. (Percent of Total Weight)

Aggregate Size Class	Sample #1	Sample #2	Sample #3	Mean and Standard Error
5 - 10 cm	35.6 %	36.0 %	48.6 %	40.1 ± 4.3
2 - 5 cm	24.4 %	34.6 %	16.4 %	25.1 ± 5.3
1 - 2 cm	20.8 %	11.5 %	13.4 %	15.2 ± 2.8
< 1 cm	19.6 %	18.1 %	22.0 %	19.9 ± 1.1

4.3.2.2 Bioremediation Suitability Assessment

In order to ascertain suitability of soil for bioremediation and to allow comparison with soils previously studied in the field, a number of organic and inorganic parameters were assessed.

Particle size distribution indicated that the soil contained 50.9 % sand and 25.7 % clay. The clay content was somewhat lower than previously encountered (36 - 44 %). SAR (0.4), pH (7.4) and EC (0.6 dS/m) were all indicative of suitable conditions for bioremediation. The organic carbon content of the soil was somewhat higher at 4.4 % compared to previous soils which ranged between 1.8 - 3.6%. Metals levels were all below CCME and very comparable to 1995 field soils except for Hg concentrations which were substantially higher at 0.17 mg/kg (vs 0.02 - 0.04 mg/kg).

Initial THB results (standard plate count) were in the 10^5 - 10^6 range which was considered adequate. Initial Microtox® results indicated greater toxicity of the WSF, EC50 ranged between 22% and 47%, compared to results from the 1995 field work with the exception of Cell C October results, refer to Table 23.

4.3.3 Experimental Design

4.3.3.1 Factorial Design

A two-level factorial design (2^2) was used to assess the effect of nutrient adjustment (NA) and aggregate size reduction (AS). The four conditions were replicated with poisoned controls to assess abiotic removals of hydrocarbons. Condition settings are detailed in Table 27. For NA, - 1 setting indicates no nutrient were added and + 1 setting indicates nutrient were adjusted. For AS, - 1 setting indicates soil was not processed and + 1 setting indicates soil was processed.

Table 27. Factorial Design for Bench-Scale Experiment.

Condition #	Condition # (poisoned control)	NA	AS
1	5	- 1	+ 1
2	6	+ 1	+ 1
3	7	- 1	- 1
4	8	+ 1	- 1

4.3.3.2 Experimental Conditions

4.3.3.2.1 Initial

Processed soil was prepared manually by pushing through a 0.5 cm sieve. Between 1300 and 1500 g of soil (dry weight) was placed in the eight 2-L beakers. $HgCl_2$ (2% dry weight) was added to Conditions #5 to #8. $HgCl_2$ was dissolved in the distilled water used to adjust moisture content to 20% by dry weight. All beakers were place on a bench top in a laboratory where normal lighting and room temperature conditions prevailed.

4.3.3.2.2 Treatment

Soil moisture content was adjusted weekly by adding distilled water. For week 1 it was adjusted to 20% by dry weight. However evaporation on a weekly basis was substantial and moisture content was subsequently adjusted to 30% by dry weight. The only deviation to the treatment schedule was for week 5 where, because of operational difficulties, water was not added.

When water was added, the soil was also mixed using a bent spatula. Soil in each beaker was turned over approximately 15 times on each mixing event.

Nutrients were added during week 1, 4 and 6 to Conditions #2, #4, #6 and #8. Two different commercial fertilizers were used to obtain molar C:N:P ratios of 100:5:1. Most of the nitrogen was added in ammonium form and phosphorus was added in phosphorus oxide (P_2O_5) form. Nutrient requirements were calculated on the basis of mean T.E.H. levels and available NO_3^--N, NH_4^+-N and PO_4^{3-}-P analysis results for each condition. Because of the typically small amounts of nutrients required (100 - 600 mg), they were dissolved in the water used to adjust soil moisture content prior to addition.

4.3.4 Analytical Methods

4.3.4.1 General

Analytical methods used for initial soil characterization and weekly analyses were similar to those used during field work and are described in section 3.3. Specific changes in analytical procedures and pertinent quality control data are included below.

4.3.4.2 Sample Identification

The following alphanumeric sample coding system was used:

ED-n-c-yymmdd

where:

-ED: two-letter code identifying soil was from the Edmonton facility;

-n: one-characterer numeric code identifying Condition #, from 1 to 8 as described in Table 27 above;

-c: letter identifying replicate for T.E.H. analysis, from A to C;

-yymmdd: six-character numeric code identifying the date on which the sample was collected (year/month/day).

4.3.4.3 Schedule

Scheduling of sampling events and analyses performed are presented in Table 28.

Table 28. Soil Sampling and Analysis Schedule for Bench-Scale Experiment.

Sampling Event	T.E.H. (C_7 - C_{30})	Soil Moisture Content	Available Nutrients	THB	Microtox®
Wk 0 (22 Nov 95)	X	X			
Wk 1 (29 Nov 95)	X	X	X	X	
Wk 2 (05 Dec 95)	X	X	X	X	
Wk 3 (12 Dec 95)	X	X	X	X	
Wk 4 (18 Dec 95)	X	X	X	X	
Wk 6 (04 Jan 96)	X	X	X	X	
Wk 8 (18 Jan 96)	X	X	X	X	X

Wk: week

4.3.4.4 T.E.H. (C_7 - C_{30}) and Soil Moisture Content

T.E.H. analysis was performed on three sub-samples for each sampling event. Sub-samples were collected in glass jars as 30 g from which 10 g was used for T.E.H. analysis and 20 g was used for soil moisture content analysis. Jars were stored at 4°C prior to analysis. For non-processed conditions care was taken to include aggregates, or parts of aggregates, of different sizes in approximately the same weight percentage as was determined from aggregate size distribution analysis.

Quality control measures for T.E.H. analysis were discussed in detail in Section 3.3.12. Method precision estimates are probably less valid here as an effort was made to obtain varied sub-samples. Nevertheless COV on triplicate analysis ranged between 6% and 62%, with a mean of 28%. Estimates of instrument precision on single compounds yielded somewhat higher results than previously. Within-sampling event COV ranged between 2% and 39%, with a mean of 14%. This was also true of estimates of instrument precision on DRO analysis. Within-sampling event COV ranged between 1% and 54%, with a mean of 21%. In order to minimize the effect of this variability on within-sampling event T.E.H. concentrations, all DRO response factors obtained during a sampling event were used to obtain the calibration curve. In order to minimize between-event variability the same standard was used throughout the eight week period. Between-sampling event COV for DRO recovery factors ranged between 31% and 36%, with a mean of 33%.

Mean recoveries for 1-chlorooctane and 1-chlorooctadecane from method blanks were 61% and 98% respectively. Mean recoveries for each sampling event were consistently greater than these minimums which was considered satisfactory.

Contamination expressed as a percentage of mean DRO area for each event was consistently smaller than 1.3% for glassware blanks and 2.1% for method blanks. This was considered to be insignificant.

COV from soil moisture content analyses ranged from 2% to 35%, with a mean of 12%. Again this estimate of method precision included sample variance.

4.3.4.5 Available Nutrients

Soil (65g, wet weight) was collected and sent to Enviro-Test Laboratories for analysis. Available nutrients analysis included NH_4^+ - N, NO_3^- - N and PO_4^{3-} - P.

4.3.4.6 THB

Soil (5g, wet weight) was collected in sterile plastic bags and stored at 4°C prior to analysis. The geometric mean of three plate counts was reported.

4.3.4.7 Microtox®

Soil (25g, wet weight) was collected in sterile plastic bags and stored at 4°C prior to analysis. Phenol standard tests performed for each batch of analyses yielded EC50 between 13 and 17 mg/L which is considered acceptable. Overall COV was 15% which was calculated including all analyses performed during the project. The analytical technique was considered to be in control.

4.4 Results and Discussion

4.4.1 T.E.H. (nC_7 - nC_{30})

4.4.1.1 General

Weekly (week 0 - 4) and biweekly (week 4 - 8) T.E.H. results are included in Table 29. For sample ED-7-C week 3 result, problem in the extraction step did not allow quantification of T.E.H. Week 0 chromatographic fingerprints indicated that hydrocarbons recovered were in the C_7 to C_{24} range indicating diesel fuel contamination, based on Restek Diesel Fuel #2 standard retention times. Figure 5 illustrates a typical chromatographic fingerprint from a week 0 sample compared to the Diesel standard.

4.4.1.2 Initial Results

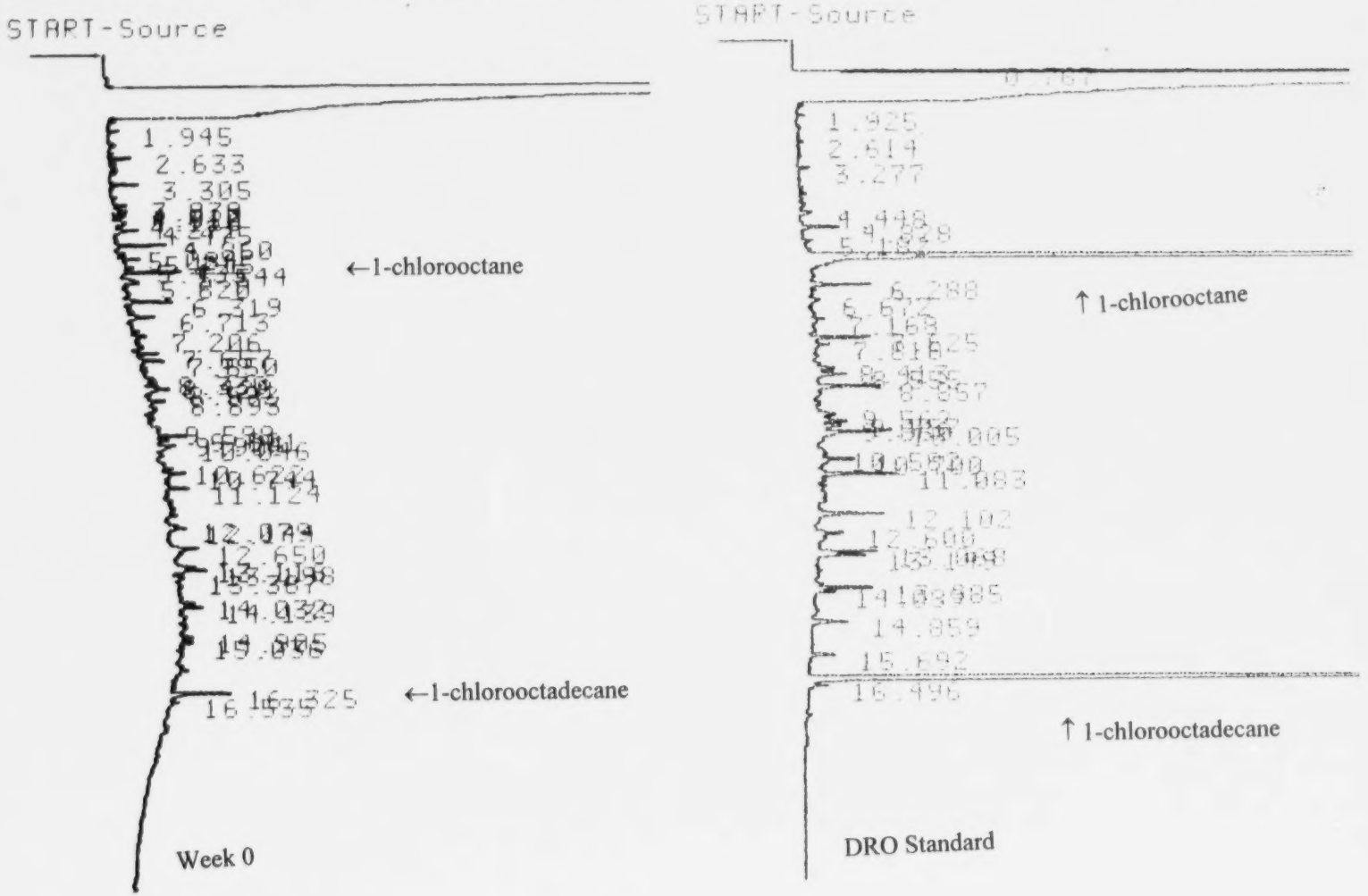
The 24 week 0 T.E.H. results were assessed for normality and it was found that the natural log of the data was normally distributed. No outliers were identified. Each condition mean was checked against mean of other 21 samples; significant differences were not found for any of the 8 conditions (t-test, α=0.05). It was therefore assumed that initial T.E.H. was similar for all conditions. For purposes of statistical evaluations of subsequent results it was also assumed that the three sample results for each condition were ln-normally distributed.

Table 29. Soil T.E.H. Analyses Results - Bench-Scale Experiment.

(X 10^1 (except as indicated) mg/kg dry soil)

Sample #	Week 0	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
ED-1-A-	584	376	332	250	574	165	134
ED-1-B-	356	450	384	694	710	171	137
ED-1-C-	439	272	487	335	248	136	117
ED-2-A-	330	445	184	115	118	44.4	48.7
ED-2-B-	395	268	185	129	76.5	74.8	54.4
ED-2-C-	414	301	150	212	212	62.3	51.7
ED-3-A-	369	236	189	118	156	132	109
ED-3-B-	700	386	272	150	169	118	118
ED-3-C-	716	510	530	285	215	109	131
ED-4-A-	707	478	223	146	88.5	81.1	48.8
ED-4-B-	388	363	203	118	119	52.1	74.2
ED-4-C-	460	566	287	235	81.2	65.6	37.2
ED-5-A-	500	295	409	234	388	157	257
ED-5-B-	400	435	273	334	767	440	176
ED-5-C-	483	430	347	480	103 X 10^2	301	258
ED-6-A-	658	336	382	261	232	289	438
ED-6-B-	357	268	280	302	515	301	191
ED-6-C-	274	309	273	341	512	215	236
ED-7-A-	312	384	466	208	154	531	257
ED-7-B-	558	193	295	325	294	194	181
ED-7-C-	628	305	403		269	321	227
ED-8-A-	462	527	375	503	315	236	577
ED-8-B-	359	591	340	208	258	179	212
ED-8-C-	385	287	480	512	543	207	220

Figure 5. Typical Chromatographic Fingerprints from Soil T.E.H. Analysis - Bench-Scale Week 0 Sample vs DRO Standard.



The effect of soil processing on hydrocarbon recovery was assessed by comparing means between processed samples and not-processed samples. It was found to be insignificant (t-test, $\alpha = 0.05$). Processing of the soil did not affect contaminant homogeneity in the short term as there was no significant differences between processed and not-processed sample variances (F-test, $\alpha = 0.05$).

4.4.1.3 Importance of Abiotic Transformations

Week 8 T.E.H. concentrations in Conditions 1 through 4 were checked against their poisoned controls and it was found that they were all significantly lower (t-test, $\alpha=0.01$). This was checked to ascertain that abiotic transformations could not be assumed to yield similar T.E.H. removal as active samples.

4.4.1.4 Factorial Design Analysis

Analysis of the factorial design is detailed at Appendix G. The results show that addition of nutrient had a significant positive effect on the extent of T.E.H. reduction within an eight-week period. Other effects and interactions were not significant.

The range of aggregate sizes for not-processed conditions were similar to that observed in the field during the 1995 remediation season and the larger aggregate sizes were in the 5 - 10 cm range in both cases. It is thought that regular tilling of the soil was most likely responsible for producing such aggregate sizes in the field. Results obtained in the bench-scale experiment indicate that there is no advantage to further processing of the soil to reduce aggregate size.

4.4.1.5 Kinetics of T.E.H. Removal

It was found that T.E.H. removal could be adequately modeled with first-order kinetics. For each condition the natural log of T.E.H. results were plotted against time and regression analysis performed on the data. Lack of fit was evaluated by comparing the mean square of the error to the mean square associated with lack of fit. For each conditions the sum of squares associated with error was estimated as the sum of the deviation squares of triplicates from all weeks. Residuals were also evaluated to check

for lack of fit. Regression analysis results are presented in Appendix H. Although there were minor weaknesses in the assumptions underlying model adequacy it was deemed to be adequate for Conditions #1 through #3.

Lack of fit was found in Condition #4 between weeks 6 and 8. For that condition there was no significant change in T.E.H. concentration during that 2-week period (t-test, α=0.05). This could be an indication that maximum extent of removal had been reached. Although bioremediation of diesel contaminated soils has been carried out to levels around 100 ppm (Jackson and Zenobia, 1994) soil factors such as organic matter content could influence such levels. As this was only observed in one condition and soils were not monitored after week 8, conclusions cannot be drawn on the significance of this observation. For Condition #4, results from week 8 were excluded from biodegradation kinetics evaluation. Typical line fit plots for Conditions #1 and #2 are shown in Figures 6 and 7.

The first-order rate constants for all conditions are presented in Table 30. These values are in agreement with rate constants associated with bioremediation of diesel-contaminated soils under aerobic conditions which can have values as high as 0.04/day (Jackson and Zenobia, 1994). These results indicate that while processing of the soil did not have an effect on the removal kinetics addition of nutrient sped up the removal of hydrocarbons by a factor of 1.4 to 2.3. Although it has been reported that the effect of adding inorganic nutrient is most obvious on the hydrocarbons that are structurally most biodegradable (Fedorak and Westlake, 1981, Dibble and Bartha, 1979), it is suggested that under mixed crude oil/petroleum products conditions the addition of inorganic nutrients can only be beneficial.

Figure 6. Bench-Scale Soil T.E.H. Removal First-Order Line Fit Plot - Condition #1

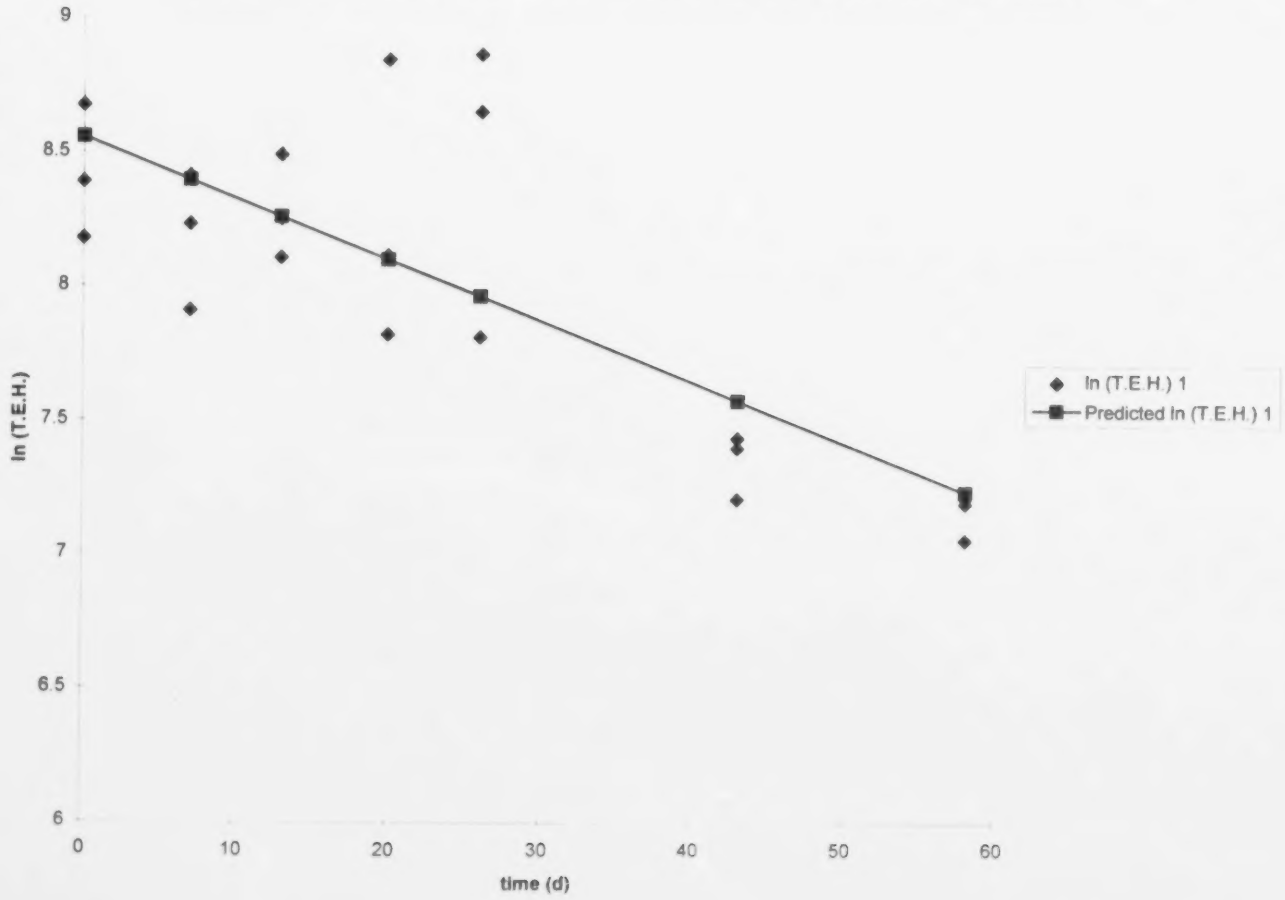


Figure 7. Bench-Scale Soil T.E.H. Removal First-Order Line Fit Plot - Condition #2

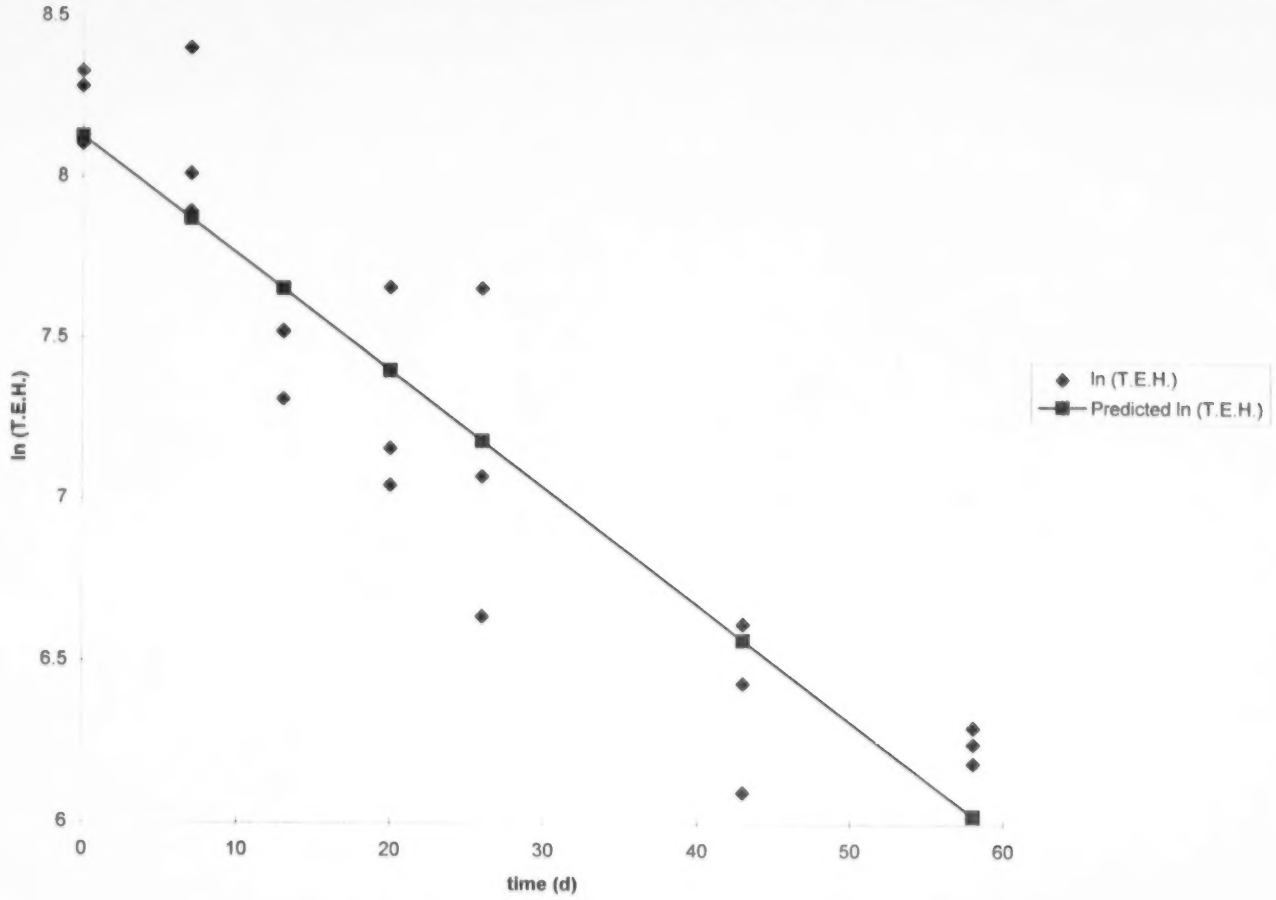


Table 30. First-Order Rate Constants for T.E.H. Removal - Bench Scale Experiment.

Condition #	k (day^{-1})	95% confidence limits
1 (no nutrient, processed)	0.023	0.014 to 0.032
2 (nutrient added, processed)	0.036	0.029 to 0.043
3 (no nutrient, not processed)	0.026	0.017 to 0.035
4 (nutrient added, not processed)	0.052	0.041 to 0.063

4.4.1.7 Effect of soil processing on heterogeneity

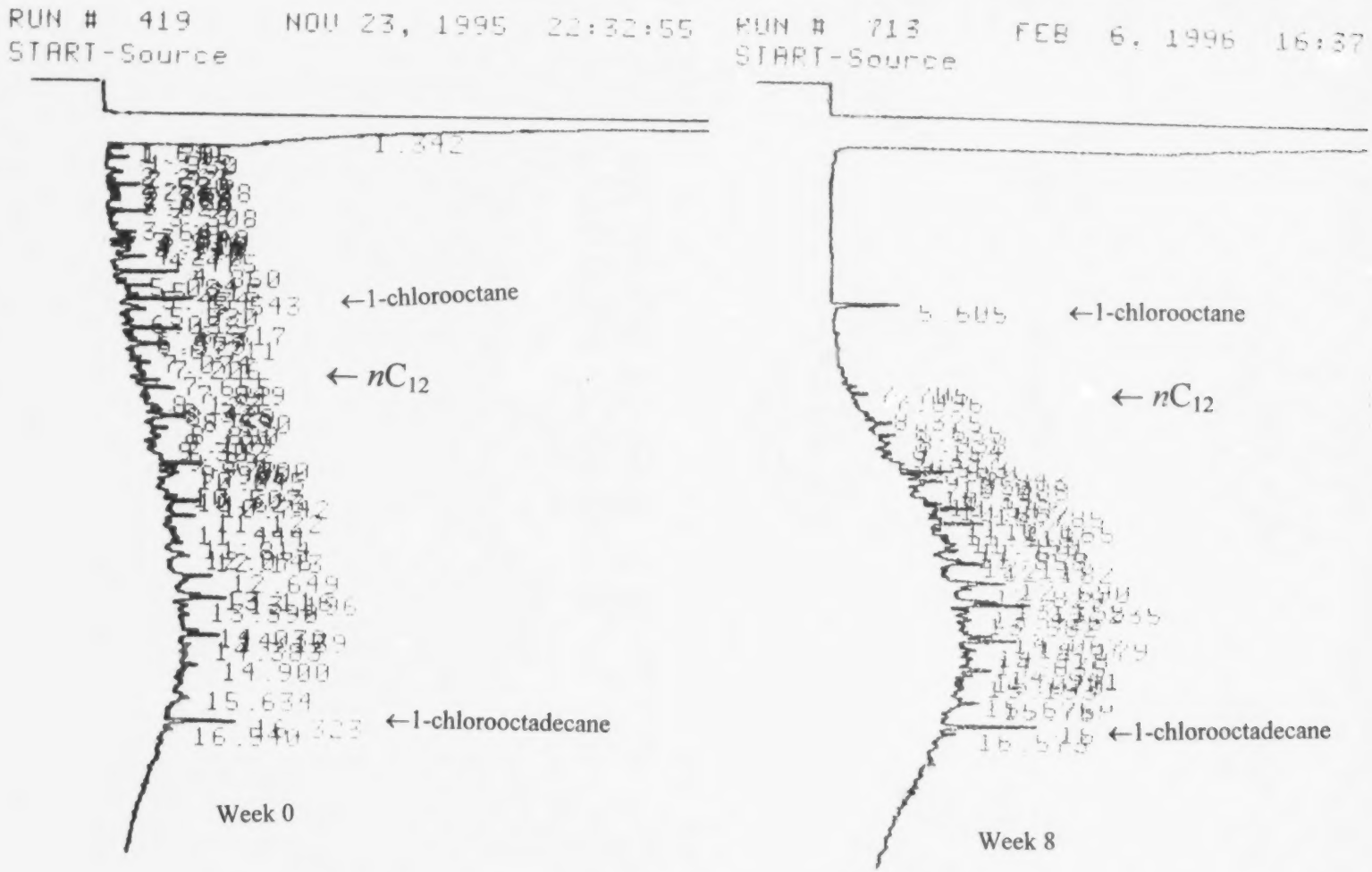
It has been observed that soil pulverization has a positive effect on reducing heterogeneity in contaminated soils (Ju et al., 1993). Final results for two sets of Conditions (#1 vs #3 and #2 vs #4) were used to verify that statement. There was no significant difference between #1 and #3 variances but #4 had a significantly larger variance than #2 (F-test, $\alpha = 0.05$). From these results the effect of aggregate size reduction on heterogeneity cannot be confirmed. This might also be due to the fact that the unprocessed soil was relatively well broken up.

4.4.1.8 Volatilization

4.4.1.8.1 General

There were significant decreases in T.E.H. levels between weeks 0 and 8 for all poisoned samples (t-test, $\alpha = 0.05$). Poisoned samples chromatographs indicated that compounds below nC_{12} were subject to removal throughout the 8 weeks. Figure 8 illustrates chromatographic fingerprints from a poisoned sample at weeks 0 and 8.

Figure 8. Typical Chromatographic Fingerprints from Soil T.E.H. Analysis for Bench-Scale Poisoned Samples - Week 0 vs Week 8



The initial fraction of hydrocarbons below nC_{12} in the poisoned samples ranged between 37% and 59% of initial T.E.H. levels. This was compared to the final T.E.H. removal in the same samples and there were no significant differences (t-test, $\alpha = 0.05$). This indicates that the removal of hydrocarbons in poisoned samples can be assumed to be caused mainly by volatilization of low molecular weight hydrocarbons. Although other removal and transformation processes were not investigated, these results indicate that their contribution would be minimal.

The extent of removal of such compounds was assessed in all samples (poisoned and non-poisoned) to investigate any possible differences. For each sample percent $< nC_{12}$ hydrocarbons was calculated from the ratio of $< nC_{12}$ area to DRO area. Percent removal for each condition was calculated for each week based on changes in mean percentage of $< nC_{12}$ hydrocarbons from three samples. Weekly percent removals are shown in Table 31. These ratios were found to be independent from DRO concentration for all weeks (correlation coefficients ranged from -0.16 to 0.40).

Table 31. $< nC_{12}$ Hydrocarbons Percent Removal for all Conditions - Bench-Scale Experiment.

(%)

Week\Condition	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
1	51.5	50.0	52.4	22.6	63.3	53.3	62.5	65.0
2	64.8	80.5	78.2	74.7	64.6	50.6	67.3	58.6
3	93.5	91.5	89.9	84.1	78.7	65.7	75.7	77.0
4	95.2	98.6	90.1	90.2	91.4	81.4	75.0	81.2
6	99.7	100	100	100	98.4	96.7	94.7	94.8
8	100	100	100	100	100	100	100	99.3

4.4.1.8.2 Initial Removal of $< nC_{12}$ Hydrocarbons

Between 50% and 65% was removed in the first week; Condition # 4 result for week 1 was not considered to be representative of most samples. There was no significant differences between poisoned and not-poisoned samples. There was no significant differences between processed and unprocessed samples indicating that the volatilization process was not promoted in the short term by processing of the soil.

4.4.1.8.3 Extent of removal of < C_{12} hydrocarbons

There was no significant difference in the percentage of < nC_{12} hydrocarbons removed from poisoned samples (99.8%) and not-poisoned samples (100%) (t-test, α = 0.05) after 8 weeks.

There was no significant difference between the percentage of < nC12 hydrocarbons removed from processed (100%) vs non-processed samples (99.8%) (t-test, α = 0.05) after 8 weeks. These results indicate that processing of the soil did not affect the extent of volatilization of < nC_{12} hydrocarbons in the long term either.

4.4.1.8.4 < C_{12} Hydrocarbons Removal Patterns

Figures 9 to 12 depict the removal patterns for < nC_{12} hydrocarbons comparing poisoned vs not-poisoned conditions. Generally, between weeks 2 and 4, weekly percent removal for not-poisoned conditions are greater than for poisoned conditions. This observation was investigated further to determine if the differences were significant.

When comparing < nC_{12} hydrocarbons removal on a weekly basis it was found that percent removals for not-poisoned conditions were greater than for poisoned conditions from week 2 to week 6 (one-sided t-test, P ranging from 0.2 to 1.6). This indicates that < nC_{12} hydrocarbons were removed faster in microbiologically active soils and it is therefore reasonable to assume that biodegradation and volatilization caused the loss of those compounds. This would support the concept of competition between volatilization and biodegradation processes proposed by Song et al. (1990).

It is therefore suggested that although final results show that reduction in T.E.H. levels between 28% and 53% occurred in poisoned samples, subtracting this loss from the loss observed in the active samples probably underestimates the true contribution of biodegradation. Another factor which could have contributed to the volatilization process in the poisoned samples is the low soil moisture status that prevailed between weeks 4 and 6, as water which was supposed to be added during

Figure 9. < *n* C12 Hydrocarbons Removal - Condition # 1 vs Condition # 5.

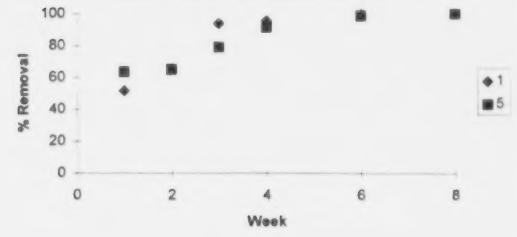
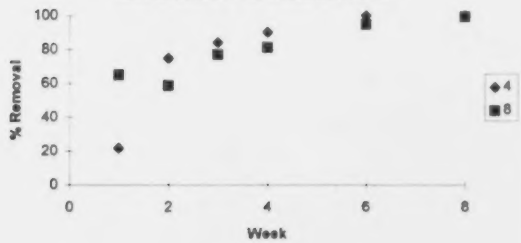


Figure 10. < *n* C12 Hydrocarbons Removal - Condition # 2 vs Condition # 6.

Figure 11. < *n* C12 Hydrocarbons Removal - Condition # 3 vs Condition # 7.

Figure 12. < *n* C12 Hydrocarbons Removal - Condition # 4 vs Condition # 8.



week 5 was not added until week 6. This would also suggest that a possible advantage of providing suitable biodegradation conditions is to minimize volatilization.

4.4.2 Soil Moisture Content

Soil moisture content analyses results are presented in Table 32 (mean of three samples). It was mentioned above that dry conditions probably prevailed between weeks 4 and 6; this is not reflected in week 6 soil moisture content results as the moisture adjustment that was supposed to be done on week 5 was done only two days prior to week 6 sampling. Week 8 results are lower and not necessarily representative of soil conditions at the time of sampling as there was a 14-day delay between sampling and analysis.

Table 32. Soil Moisture Content Analyses Results- Bench-Scale Experiment.
(% w/w dry soil)

Week	C. # 1	C. # 2	C. # 3	C. # 4	C. # 5	C. # 6	C. # 7	C. # 8
0	19	19	21	17	21	19	22	20
1	11	11	11	13	11	11	8	10
2	18	17	14	16	17	19	16	13
3	15	18	17	14	17	20	18	17
4	16	15	17	18	16	17	15	12
6	17	14	17	15	13	14	14	16
8	7	7	11	8	6	7	9	10

(C.: condition)

4.4.3 THB

All THB results for poisoned samples resulted in zero counts, estimated on the 1:100 soil : peptone water dilutions, confirming little or no microbial degradation occurred in these samples. THB results for Conditions #1 through #4 are included in Table 33. Mean and standard error of three replicates are included.

Analysis of variance was performed on the weekly results using Microsoft® Excel 7.0 statistical analysis package. It was found that there were some significant differences among conditions in weeks 2 to 8 ($\alpha \leq 0.02$). From weeks 2 to 8, mean concentrations for Condition # 2 were consistently higher than those for Condition # 1. Similarly mean

concentrations for Condition #4 were consistently higher than those for Condition # 3 for weeks 3 to 8. These results are expected and indicate that the greater removal rate of hydrocarbons in Conditions #2 and #4 was supported by a larger microbial population.

Table 33. THB Analyses Results - Bench-Scale Experiment.
(CFU / g soil)

Week	Condition # 1	Condition # 2	Condition # 3	Condition # 4
1	4.35E5 ± 0.64E5	3.60E5 ± 0.36E5	3.93E5 ± 0.70E5	4.76E5 ± 1.18E5
2	3.11E5 ± 4.74E5	2.65E6 ± 0.21E6	4.12E5 ± 1.95E5	4.82E5 ± 2.92E5
3	8.32E4 ± 1.80E4	1.57E5 ± 0.40E5	2.70E5 ± 0.12E5	5.81E5 ± 0.40E5
4	8.14E5 ± 1.28E5	2.04E6 ± 0.23E6	9.17E5 ± 1.30E5	3.98E6 ± 0.30E6
6	5.24E5 ± 0.73E5	3.03E6 ± 0.35E6	1.15E6 ± 0.24E6	5.48E6 ± 0.98E6
8	2.29E5 ± 1.30E5	8.71E5 ± 1.74E5	6.95E5 ± 5.31E5	3.87E6 ± 0.62E6

4.4.4 Available Nutrients

4.4.4.1 Nutrient Availability

Nitrate-N and ammonia-N results for poisoned samples are considered to be invalid because of Hg interference with analytical methods (APHA, 1994). Nitrate-N results are presented in Table 34. Nitrate-N levels stayed relatively constant for all conditions except for Condition #4 where there was an increase in the last two weeks. It is suggested that, towards the end of the study period, added ammonium was no longer necessary for microbial utilization of hydrocarbons and was therefore subject to nitrification. This is supported by the lack of significant T.E.H. removal in Condition # 4 discussed in section 4.4.1.5. This would suggest that, when inorganic nutrients are added in ammonia-N form, increases in nitrate levels might be an indication of minimal microbial degradation of hydrocarbons.

Table 34. Soil Nitrate-N Analyses Results - Bench-Scale Experiment.
(mg/kg dry soil)

Week	Condition # 1	Condition # 2	Condition # 3	Condition # 4
1	1.8	1.0	1.0	1.2
2	5.2	1.4	1.2	0.8
3	1.0	0.6	0.6	0.6
4	0.2	0.2	0.2	0.4
6	0.8	2.2	0.4	15.2
8	0.6	1.2	0.6	71.0

Ammonium-N results are presented in Table 35. There are no distinguishable trends in ammonium-N levels for Conditions #1 and #3. Although results for Conditions #2 and #4 indicate a high variability, there are also indications that added ammonium-N is being utilized.

Table 35. Soil Ammonium-N Analyses Results - Bench-Scale Experiment.
(mg/kg dry soil)

Week	Condition # 1	Condition # 2	Condition # 3	Condition # 4
1	1.2	31.2	1.4	109.0
2	1.0	2.0	7.6	3.8
3	0.6	0.8	0.6	21.8
4	6.0	12.6	4.6	32.0
6	1.0	5.4	0.8	50.0
8	1.0	1.6	1.0	2.4

It is interesting to note that both nitrate-N and ammonium-N levels remained constant for Conditions #1 and #3. Obviously the soil nutrient content was adequate to sustain microbial degradation of hydrocarbons. However the increased degradation rate produced by the addition of more readily available inorganic nutrient suggests the utilization of soil nutrient content might be a rate-limiting factor.

Orthophosphate levels are presented in Table 36. There are no apparent decreasing trends for Conditions #1 and #3. Levels for Conditions #2, #4, #6 and #8

indicate the potential for high variability in available phosphorus results when inorganic phosphorus is added.

Table 36. Soil Orthophosphate-P Analyses Results - Bench-Scale Experiment. (mg/kg dry soil)

Week	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8
1	2.9	15.9	1.3	16.8	0.7	15.4	0.6	9.5
2	1.5	5.2	1.1	8.4	0.9	7.2	2.0	4.2
3	1.2	5.6	1.0	18.4	1.8	8.2	0.6	3.3
4	1.3	5.6	0.7	17.0	1.4	12.0	1.3	23.4
6	1.2	14.1	0.9	18.1	1.4	20.2	0.9	7.5
8	2.2	26.2	1.6	20.8	18.0	2.2	2.0	8.0

Weekly C:N:P ratios were calculated for Conditions # 2 and # 4 and results are presented in Table 37. These results indicate that based on available nutrient analyses results, a proper C:N:P ratio was never attained, except for Condition # 4 on weeks 6 and 8. However T.E.H. results have shown that the bioremediation process was significantly promoted for these conditions. It is suggested that the C:N:P ratio does not provide a proper indication of the suitability of the soil conditions to sustain an optimal bioremediation process. These findings are in accordance with information presented at the 5th Annual Symposium on Groundwater and Soil Remediation where it was stated that the C:N:P fixed ratio rule was found to be invalid (Fang, 1995).

Table 37. Soil C:N:P Ratios - Bench-Scale Experiment.

Week	Condition # 2	Condition # 4
0	100 : 0.06 : 0.02	100 : 0.05 : 0.01
1	100 : 0.82 : 0.18	100 : 2.01 : 0.14
2	100 : 0.17 : 0.12	100 : 0.17 : 0.14
3	100 : 0.08 : 0.14	100 : 1.15 : 0.43
4	100 : 0.81 : 0.16	100 : 2.88 : 0.68
6	100 : 1.08 : 0.90	100 : 8.43 : 1.06
8	100 : 0.47 : 1.22	100 : 11.79 : 1.51

4.4.4.2 Nutrient Utilization

In order to assess nutrient utilization the amounts of nitrogen and phosphorus added were compared to total available N (nitrate-N and ammonia-N) and orthophosphates analyses results. Figure 13 shows theoretical N concentrations as bars and reported N concentrations as x-y plots for Conditions #2 and #4. Similarly Figure 14 pertains to phosphorus concentrations. For weeks 1 and 4 nutrients were added 4 days prior to analysis and for week 6 they were added 15 days prior to analysis. It can be seen that there are considerable differences between theoretical and reported N and P concentrations. The differences can be attributed to poor nutrient mixing causing heterogeneity and nutrient utilization and/or fixing. For these reasons it does not seem adequate to make conclusions on nutrient utilization rates.

As discussed in section 4.4.1.4, this study indicates that addition of inorganic nutrient causes an increase in degradation rates. However it has been shown that available nutrient analyses results are not necessarily a good indication of nutrient requirements and do not provide information on nutrient utilization rates. It is suggested that nutrient requirements might be better estimated based solely on hydrocarbon content. This study indicates that when calculating total nutrient requirements based on mean initial T.E.H. levels, a ratio of 6 mol N and 1.4 mol P to 100 mol C produced ideal conditions. This was calculated on the assumption of 85% carbon content (Riser-Roberts, 1992). Conversion efficiency was not taken into account because of the very wide range of

conversion efficiencies offered in the literature; this in effect produces a safety margin in the amount of nutrients added.

Figure 13. Bench-Scale Total Soil N Levels for Conditions #2 and #4 - Theoretical vs Reported

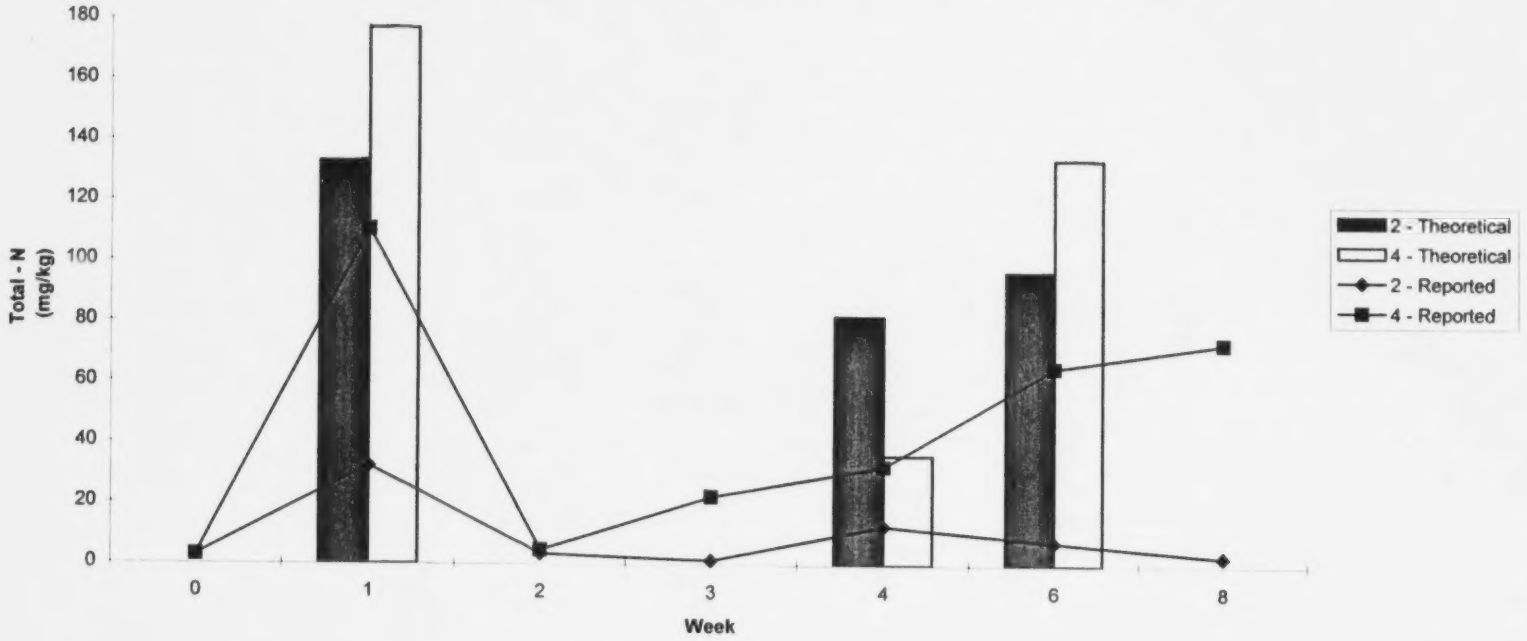
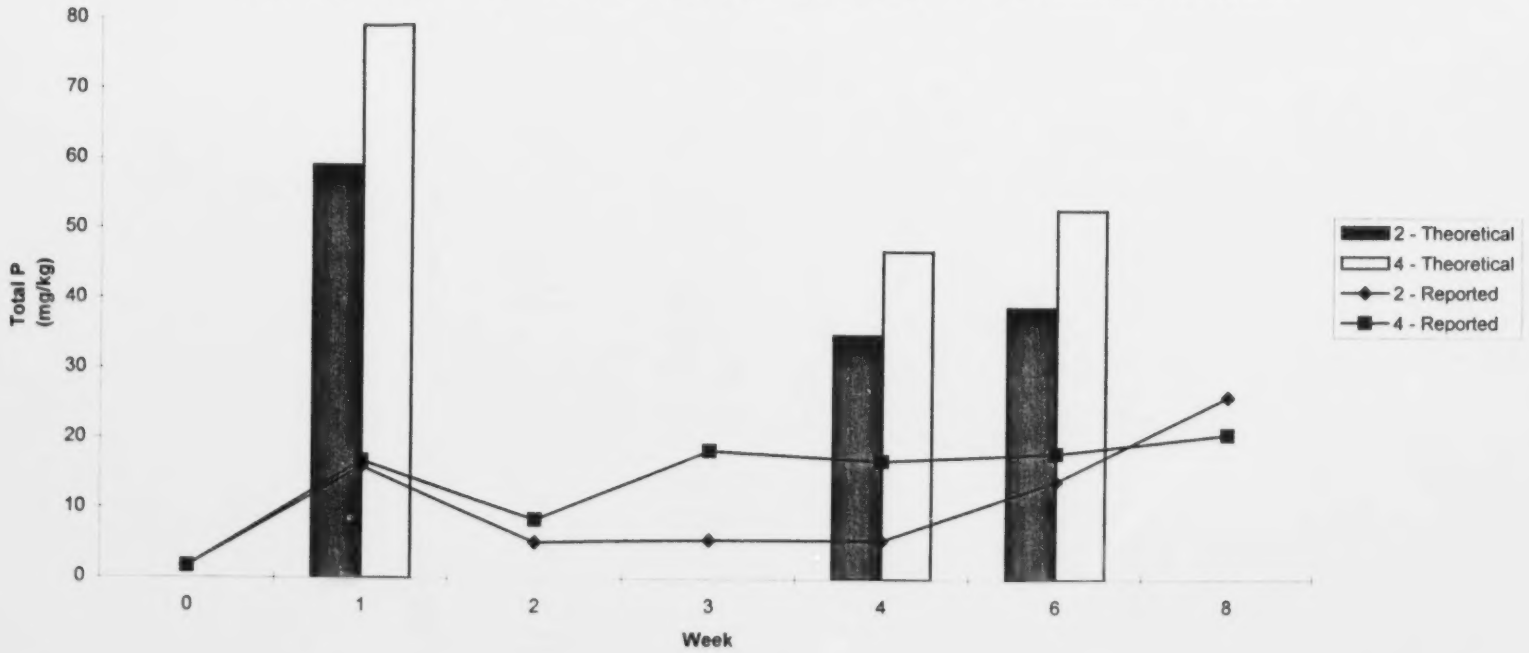


Figure 14. Bench-Scale Total Soil P Levels for Conditions #2 and #4 - Theoretical vs Reported



4.4.5 Microtox®

Initial and final EC50 are reported in Table 38. Inital analyses were performed on three replicates, indicated by A, B or C, each from the not-processed soils and processed soils. Final EC50 for Conditions #5 through #8 could not be assessed because of the presence of $HgCl_2$. Initial results show that there were no apparent differences between processed and not-processed samples. Final results indicate that detoxification occurred but there were no apparent differences between conditions. This indicates that in terms of WSF toxicity, 500 ppm vs 1000 ppm residual hydrocarbon levels are similar. The relatively high toxicity is indicative of the high solubility of the hydrocarbons remaining.

Table 38. WSF Microtox® Analyses Results - Bench-Scale Experiment.
(EC50, 5 min)
(all results are as % concentration of undiluted WSF)

	Sample	Lower 95% C.L.	EC 50 (%)	Higher 95% C.L.
Initial	Not-Processed-A	43	47	52
	Not-Processed-B	19	23	28
	Not-Processed-C	20	25	32
	Processed-A	18	22	26
	Processed-B	21	26	31
	Processed-C	19	33	55
Final	ED-1-960118	40	54	71
	ED-2-960118	38	74	145
	ED-3-960118		> 100	
	ED-4-960118	37	52	73

It is interesting to compare these toxicity results to toxicity results obtained in May 1995 where mean T.E.H. concentrations as high as 2500 ppm for Cell C yielded

EC50's greater than 100% indicating very low toxicity. The major difference between the bench-scale samples and the field samples is the range of hydrocarbons concerned. While bench-scale samples contained more soluble hydrocarbons between C_{12} to C_{24}, field samples were contaminated with hydrocarbons in the C_{14} to $C_{>30}$ range. It should also be noted that there might be other removal mechanisms such as run-off from rain events contributing to the faster removal of the soluble hydrocarbons under field conditions.

This observation highlights the fact that in terms of remediation end point, when considering toxicity of the WSF a numerical value based on T.E.H. levels is not necessarily representative of "risk-free" conditions. Numerical remediation guidelines might be better set on the basis of a narrow range of hydrocarbons rather than on a wide range such as T.E.H. (C_7 - C_{30}).

5.0 Conclusions and Recommendations for Further Studies

5.1 Conclusions

Conclusions pertaining to the field work are presented here.

1. All soils at the Edmonton RBF were successfully remediated throughout the 1995 season. There were decreases in mean T.E.H. levels of 65% in Cell C and 59% in Cell D between May and July 1995. Cell A did not experience a significant reduction in T.E.H. levels during that same period. However October results indicated a 72% decrease in T.E.H. levels for Cell A between July and October 1995. Soil conditions, treatment operations or chromatographic fingerprint analyses did not indicate differences in Cell A that could have caused the apparent retardation in biodegradation.
2. Similar results were found for the new soils placed in Cells C and D with October results indicating decreases in mean T.E.H. concentrations of 55% for Cell C and 51% for Cell D between 13 Sep 1995 and 17 Oct 1995.
3. Based on first-order kinetics modeling, hydrocarbon removal rates observed at the Edmonton RBF in 1995 were within the range or somewhat higher than rates reported in the literature for full-scale bioremediation of medium molecular weight petroleum contamination.
4. Combined decreases in the variability and value of hydrocarbon concentrations were observed. This homogenization effect has been observed in other systems and is believed to be a combination of mixing effect from tilling of the soil and bioremediation.
5. Field results indicate that the high clay content of the soils, between 35.9% and 44.4%, and TOC levels between 1.8% and 3.6% did not seem to affect the bioremediation process.
6. July results indicated that although the T.E.H. levels were significantly higher in Cell A, the toxicity levels of the WSF were similar in all three cells. As well the higher toxicity observed in the new soil from Cell C in October was associated with lower

T.E.H. concentrations compared to May T.E.H. levels for which there was no toxicity detected. Numerical T.E.H. level remediation goals do not guarantee toxicity-freeWSF.

7. The major difference between 1994 and 1995 treatment operations was the tilling frequency. This is believed to have contributed to the good performance of the process in the following ways. It was critical in controlling the moisture content in the cells and therefore ensuring oxygen availability. It was also instrumental in producing the much smaller soil aggregates observed in 1995 again ensuring oxygen availability. Tilling action also redistributes the oil, nutrients and microorganisms within the soil system.

Conclusions pertaining to the bench-scale optimization experiment are presented below.

8. Addition of inorganic nutrient had a significant positive effect on the extent of T.E.H. reduction within an 8-week period. Specifically it increased the rate of removal of diesel range hydrocarbons by a factor of 1.4 to 2.3.
9. There is no advantage to further processing of the soil to reduce aggregate size from about 5 to 7 cm in diameter down to 0.5 cm in diameter.
10. Degradation of diesel range hydrocarbons by indigenous microorganisms can be adequately modeled with first-order kinetics in the concentration range studied (less than 10 000 mg/kg T.E.H. (C_7 - C_{30}).
11. The C:N:P ratio as calculated from mean T.E.H. concentration and available nutrient levels does not provide a proper indication of the suitability of the soil conditions to sustain an optimal bioremediation process.
12. In terms of WSF toxicity, 500 ppm vs 1000 ppm residual hydrocarbon levels are similar. The relatively high toxicity is indicative of the high solubility of the hydrocarbons remaining and not based on the concentration level.

5.2 Recommendations for Further Studies

The recommendations for further studies which are made here pertain specifically to the operation of IPL's RBF as well to the general topic of hydrocarbon contaminated soil remediation.

1. It was identified that a standard analytical method for hydrocarbon contaminated soils has not been specified neither by the agency regulating the operation of IPL's RBF, being the National Energy Board, nor by other regulatory bodies such as CCME (CCME,1993). Simply there is a need for definition of "what should be analyzed for" i.e. specifying proper analytical parameter(s) and "how it should be analyzed" i.e. specifying analytical method(s) for those parameter(s). The practice of specifying broad analytical parameters such as T.E.H. must be reviewed as it has been stated that if used in isolation rather than as part of a disperse analytical framework, these methods present a greatly over-simplified picture of the nature and distribution of contaminants which can lead to potentially damaging and expensive miscalculation during remediation (Whittaker et al., 1995). Although the GC-FID method used in this study has been described in the literature (Sauer and Boehm, 1991), there is still a need to define appropriate standards that can be used in the quantification of hydrocarbon concentrations in extracts.
2. In this study, Microtox® analyses performed on the WSF of the contaminated soils allowed for a relative assessment of toxicity associated with remediated soils. As was stated above, the relative toxicity of the WSF is not positively correlated with T.E.H. concentrations i.e. a low T.E.H. concentration is not necessarily associated with a low toxicity value. Rather it seems that the level of toxicity is associated with contaminant characteristics, namely solubility. The fact that in terms of remediation end point, when considering toxicity of the WSF, a numerical value based on T.E.H. conentration levels is not necessarily representative of "risk-free" conditions needs to be highlighted. Numerical remediation guidelines might be better set on the basis of a narrow range of hydrocarbons of similar characteristics rather than on a wide range such as T.E.H. (C_7 - C_{30}).

3. For monitoring purposes, increasing the number of samples taken from each cell would contribute to reducing the magnitude of the confidence limits on the mean T.E.H. levels therefore minimizing the effects of contaminant heterogeneity on the remediation effort. For this reason, the sampling scheme presently used might be reviewed.
4. There is a need for a way of classifying IPL's contaminated soils with respect to approximate soil contamination levels so that soils which are already below the remediation limit can be handled with appropriately.
5. Major difficulties were encountered in the analysis of soil phenolic compounds in this study. CCME has recommended EPA method 8270B for phenolic compounds analysis as the only generally applicable method that is recommended for use with soils and sediments (CCME, 1993). It is suggested that the analytical method used to analyze for soil phenolic compounds be reviewed.
6. The requirement for proper moisture control was discussed both within the context of field work and bench-scale optimization experiment results. While weather conditions cannot be controlled, drainage problems within the cells should be prevented. The high clay content of some of the soils treated at IPL's RBF most likely compounds any drainage problems. As all types of soils must be treated efficiently it is essential that drainage performance be optimal. Design specifications for the construction of the cells (Hardy BBT Limited, 1991) should be reviewed to ensure they are still met.

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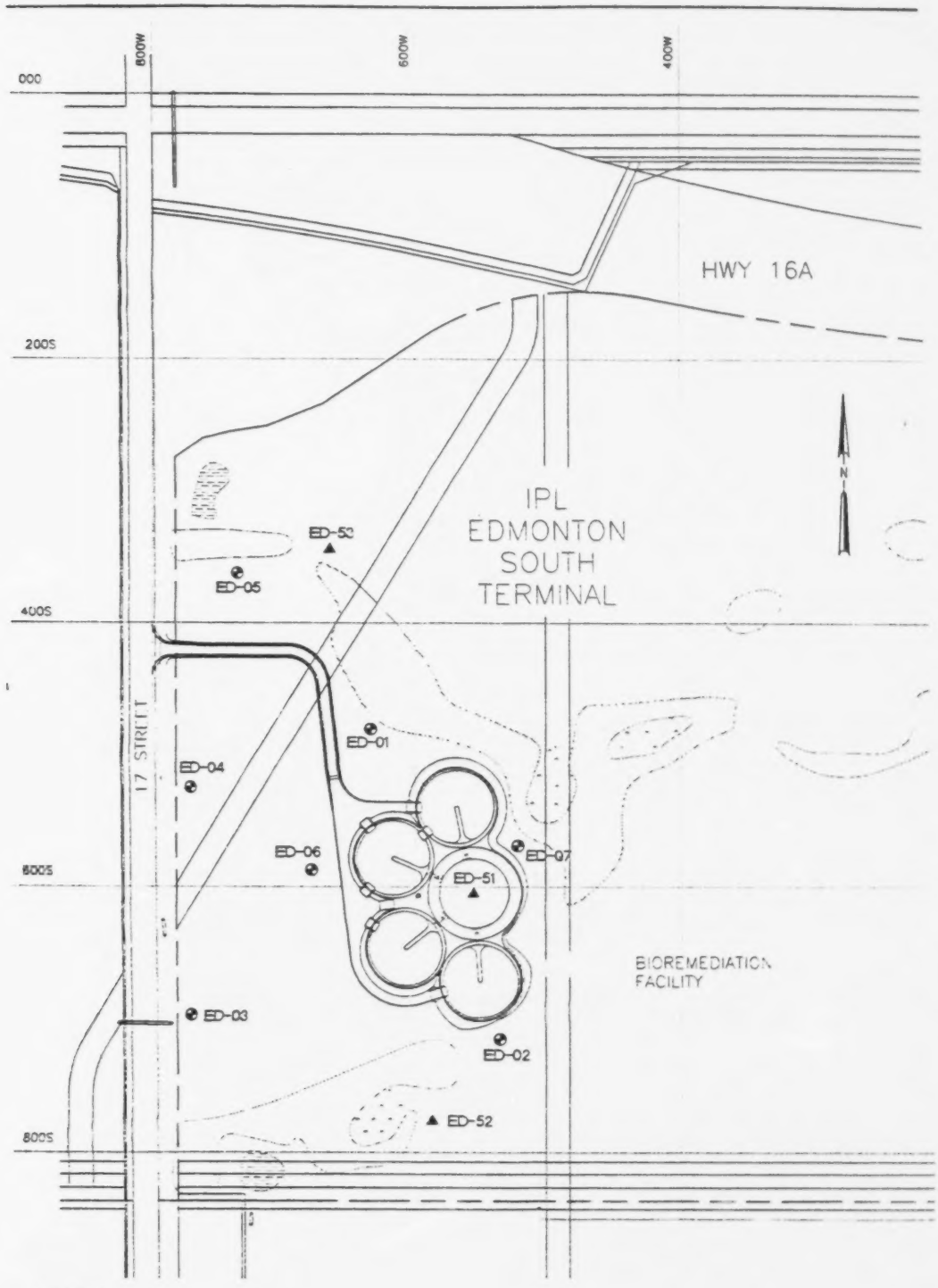
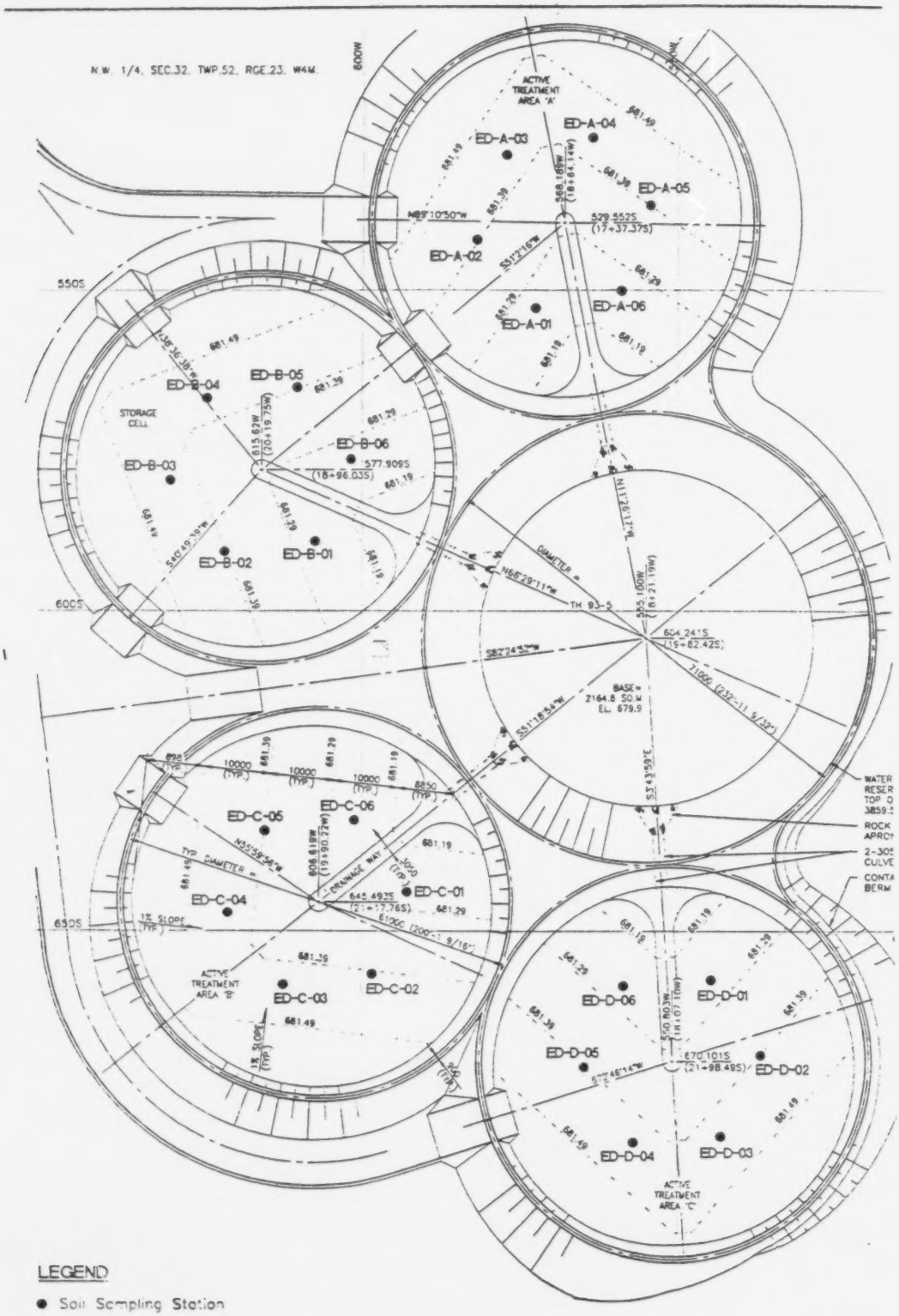
APPENDIX A - Edmonton Regional Bioremediation Facility Layout

Ref: CH2M Hill, 1994.



LEGEND

▲ Surface Water Sampling Station
◕ Groundwater Sampling Station



LEGEND

● Soil Sampling Station

APPENDIX B - Field Reporting Forms

Field Activities /Observations Report

Site Location: Edmonton, AB
Site Description: RBF

Date: 24/5/95
25/5/95

Weather: windy, overcast Temp: ~10 C Time: 0930 - 12 30
0915 -

Sampling Parameters: 18 grab samples
3 composite samples
8-10 water samples

Sampling Performed By: MC, Fauzal, IPL Co-op student

Decon Performed By: Fauzal

Required Sampling Equipment:
21 plastic bags
augers
measuring tapes
~~Measuring tapes~~
gloves (latex)
Tyvek suits
paper towels
shovel
water level meter
tape
water sampler
Key/calculator

Required Decontamination Equipment:
distilled water
isopropanol
detergent
pails
gloves (nitrile)
safety glasses
face shield
brush

Required Decontamination Procedure:
soil - scrape excess soil
wash w/ detergent, rinse w/ d.w.
wash w/ isopropanol, rinse w/ d.w.
air dry

Decon Location:
water:
- detergent
- rinse w/ d.w.
- source water.

Required Containment/Disposal of Decon:
liquid run-off pond
solid on site

Misc. Notes:

Soil Sample Description

Cell: A - NOT A CIRCLE Date: 25/25/95

Sample ID #	Visual Description
01	Depth: shallow from side center ~10-15cm Odor: same - wet soil Texture: same Color: dark brown - moisture Moisture: rain event previous day Other:
02	Depth: 15 - 30 cm Odor: Texture: Color: Moisture: Other:
03	Depth: Odor: Texture: Color: Moisture: Other:
04	Depth: 15-30cm Odor: Texture: Color: Moisture: Other:
05	Depth: Odor: Texture: Color: rust color Moisture: Other:
06	Depth: Odor: Texture: Color: Moisture: Other:
	Depth: Odor: Texture: Color: Moisture: Other:

Misc. Notes: * all samples (A, C, D) not homogeneous throughout length.

APPENDIX C - Detailed Analytical Results - T.E.H. and Phenols

Table 1. Detailed Analytical Results - TEH - May 1995

SAMPLE #	T.E.H. (mg/kg) per dry weight					
Replicates	1	2	3	mean	st dev	COV (%)
ED-A-01-950525	1981			1981		
ED-A-02-950525	2157			2157		
ED-A-03-950525	5031			5031		
ED-A-04-950525	2584	2820	1688	2364	597	25.26
ED-A-05-950525	1389			1389		
ED-A-06-950525	304			304		
ED-A-07-950525	6034	2223		4129	2695	65.27
ED-C-01-950524	5183			5183		
ED-C-02-950524	1883			1883		
ED-C-03-950524	2535			2535		
ED-C-04-950524	1001	1182	1321	1168	160	13.74
ED-C-05-950524	4056	1465	3745	3089	1415	45.80
ED-C-06-950524	1153			1153		
ED-C-07-950524	2577	2014	1929	2173	352	16.20
ED-D-01-950524	1180			1180		
ED-D-02-950524	3351			3351		
ED-D-03-950524	1880	1787	3364	2344	885	37.76
ED-D-04-950524	694			694		
ED-D-05-950524	818			818		
ED-D-06-950524	374			374		
ED-D-07-950524	1212	1016	1262	1163	130	11.18

Table 2. Detailed Analytical Results - TEH - July 1995

SAMPLE #	T.E.H. (mg/kg) per dry weight					
Replicates	1	2	3	mean	st dev	COV (%)
ED-A-01-950724	965	424	448	612	306	49.92
ED-A-02-950724	6174			6174		
ED-A-03-950724	2487			2487		
ED-A-04-950724	916			916		
ED-A-05-950724	1406	951	795	1051	317	30.22
ED-A-06-950724	1682			1682		
ED-A-07-950724	959			959		
ED-C-01-950724	568			568		
ED-C-02-950724	1252			1252		
ED-C-03-950724	575			575		
ED-C-04-950724	1374	1643	857	1291	399	30.93
ED-C-05-950724	407	529	393	443	75	16.89
ED-C-06-950724	1122			1122		
ED-C-07-950724	743			743		
ED-D-01-950724	701			701		
ED-D-02-950724	426			426		
ED-D-03-950724	574	622	488	561	68	12.09
ED-D-04-950724	715	821	785	774	54	6.97
ED-D-05-950724	788			788		
ED-D-06-950724	837			837		
ED-D-07-950724	641			641		

Table 3. Detailed Analytical Results - TEH - September 1995

SAMPLE #	T.E.H. (mg/kg) per dry weight					
Replicates	1	2	3	mean	st dev	COV (%)
ED-C-01-950913	985			985		
ED-C-02-950913	972			972		
ED-C-03-950913	1247			1247		
ED-C-04-950913	156	295	426	292	135	46.19
ED-C-05-950913	10850			10850		
ED-C-06-950913	582	422	405	470	98	20.79
ED-C-07-950913	9521			9521		
ED-D-01-950913	946			946		
ED-D-02-950913	569	849	1250	889	342	38.49
ED-D-03-950913	1715			1715		
ED-D-04-950913	1828			1828		
ED-D-05-950913	1080	1708	1257	1348	324	24.02
ED-D-06-950913	153			153		
ED-D-07-950913	1025			1025		

Table 4. Detailed Analytical Results - TEH - October 1995

SAMPLE #	T.E.H. (mg/kg) per dry weight					
Replicates	1	2	3	mean	st dev	COV (%)
ED-A-01-951017	288	315	293	299	14	4.81
ED-A-02-951017	386	369	347	367	20	5.32
ED-A-03-951017	449			449		
ED-A-04-951017	838			838		
ED-A-05-951017	1019			1019		
ED-A-06-951017	631			631		
ED-A-07-951017	479			479		
ED-C-01-951017	350	361	321	344	21	6.01
ED-C-02-951017	550			550		
ED-C-03-951017	597			597		
ED-C-04-951017	778	867	765	803	56	6.91
ED-C-05-951017	3264			3264		
ED-C-06-951017	1181			1181		
ED-C-07-951017	820			820		
ED-D-01-951017	636			636		
ED-D-02-951017	482	448	596	509	78	15.24
ED-D-03-951017	299	370	311	327	38	11.63
ED-D-04-951017	919			919		
ED-D-05-951017	563			563		
ED-D-06-951017	449			449		
ED-D-07-951017	392			392		

Table 5. Detailed Analytical Results - Phenols - May 1995

SAMPLE	Phenol Level (ug)	Phenol Concentration (ug/kg)	Mean Sample Concentration (ug/kg)	Mean Cell Concentration (ug/kg)	Cell St Dev (ug/kg)	COV (%)
ED-A-01-950525	10.21	204.20	217	180	111	62
	11.26	225.20				
	11.02	220.40				
ED-A-02-950525	12.29	245.06	255			
	12.7	253.24				
	13.44	268.00				
ED-A-03-950525	15.79	316.94	341			
	18.17	364.71				
	16.98	340.83				
ED-A-04-950525	4.91	98.26	99			
	5.16	103.26				
	4.74	94.86				
ED-A-05-950525	5.06	101.22	126			
	6.54	130.83				
	7.3	146.03				
ED-A-06-950525	1.62	32.46	41			
	1.88	37.67				
	2.6	52.09				
ED-C-01-950524	11.26	225.65	222	604	626	104
	11.01	220.64				
	10.89	218.24				
ED-C-02-950524	10.4	207.96	186			
	8.31	166.17				
	9.27	185.36				
ED-C-03-950524	25.54	842.90	895			
	26.98	890.43				
	28.82	951.16				
ED-C-04-950524	80.98	1594.09	1613			
	82.3	1620.08				
	82.55	1625.00				
ED-C-05-950524	70.91	1398.62	1396			
	70.5	1390.53				
	70.92	1398.82				
ED-C-06-950524	15.06	301.02	323			
	16.97	339.20				
	16.47	329.20				
ED-D-01-950524	60.51	1210.20	1227	706	497	70
	61.52	1230.40				
	61.95	1239.00				
ED-D-02-950524	48.19	965.15	973			
	49.14	984.18				
	48.49	971.16				
ED-D-03-950524	7.53	150.84	140			
	6.58	131.81				
	6.86	137.42				
ED-D-04-950524	32.48	649.21	663			
	34.3	685.59				
	32.78	655.21				
ED-D-05-950524	57.21	1142.60	1143			
	56.19	1122.23				
	58.33	1164.97				
ED-D-06-950524	5	99.03	90			
	4.84	95.86				
	3.83	75.86				

Table 6. Detailed Analytical Results - Phenols - July 1995

SAMPLE	Phenol Level (ug)	Phenol Concentration (ug/kg)	Mean Sample Concentration 9ug/kg)	Mean Cell Concentration	Cell St Dev (ug/kg)	COV (%)
ED-A-01-950724	2.57	102.53	121	107	88	83
	3.2	127.67				
	3.32	132.45				
ED-A-02-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-A-03-950724	6.34	253.86	175			
	3.34	133.74				
	3.43	137.34				
ED-A-04-950724	3.83	152.25	136			
	3.26	129.59				
	3.19	126.81				
ED-A-05-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-A-06-950724	5.43	215.05	208			
	5.08	201.19				
	5.26	208.31				
ED-C-01-950724	0	0.00	0	25	50	198
	0	0.00				
	0	0.00				
ED-C-02-950724	3.31	131.17	124			
	2.96	117.30				
	3.08	122.05				
ED-C-03-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-C-04-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-C-05-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-C-06-950724	0.25	9.88	3			
	0	0.00				
	0	0.00				
ED-D-01-950724	0	0.00	0	85	123	144
	0	0.00				
	0	0.00				
ED-D-02-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-D-03-950724	212.63	8449.47	8476			
	213.24	8473.71				
	214.03	8505.10				
ED-D-04-950724	0	0.00	0			
	0	0.00				
	0	0.00				
ED-D-05-950724	4.24	168.87	159			
	4.16	165.68				
	3.55	141.39				
ED-D-06-950724	6.33	251.06	268			
	6.9	273.67				
	7.02	278.43				

Table 7. Detailed Anatytical Results - Phenols - October 1995

SAMPLE	Phenol Level (ug)	Phenol Concentration (ug/kg)	Mean Sample Concentration (ug/kg)	Mean Cell Concentration (ug/kg)	Cell St Dev (ug/kg)	COV (%)
ED-A-01-951019	0	0.00	0	309	393	127
	0	0.00				
	0	0.00				
ED-A-02-951019	9.89	365.08	371			
	9.46	349.21				
	10.77	397.56				
ED-A-03-951019	12.7	491.68	512			
	14.27	552.46				
	12.72	492.45				
ED-A-04-951019	0	0.00	0			
	0	0.00				
	0	0.00				
ED-A-05-951019	0	0.00	0			
	0	0.00				
	0	0.00				
ED-A-06-951019	24.55	951.55	972			
	24.38	944.96				
	26.32	1020.16				
ED-C-01-951019	3.71	143.80	142	410	297	72
	3.98	138.19				
	3.72	144.19				
ED-C-02-951019	21.23	803.25	784			
	20.76	785.47				
	20.19	763.90				
ED-C-03-951019	0	0.00	0			
	0	0.00				
	0	0.00				
ED-C-04-951019	8.42	329.55	348			
	10.47	409.78				
	7.82	306.07				
ED-C-05-951019	12.11	474.34	487			
	12.68	496.67				
	12.53	490.80				
ED-C-06-951019	16.33	626.15	635			
	16.75	642.25				
	16.6	636.50				
ED-D-01-951019	5.49	216.31	208	455	359	79
	5.21	205.28				
	5.17	203.70				
ED-D-02-951019	10.74	426.36	459			
	12.41	492.66				
	11.52	457.32				
ED-D-03-951019	1.92	69.21	79			
	1.79	64.53				
	2.86	103.10				
ED-D-04-951019	19.66	759.07	774			
	20.42	788.42				
	20.07	774.90				
ED-D-05-951019	22.02	864.89	836			
	20.78	816.18				
	21.06	827.18				
ED-D-06-951019	0	0.00	0			
	0	0.00				
	0	0.00				

APPENDIX D - T.E.H. Calculations

Sample ED-C-01-950724 was used to provide a sample calculation. The Integration Report pertaining to this sample can be found at the end of this Appendix.

1) Obtain Total Area, ClC_8 Area, ClC_{18} Area, Area less than C_7 and Area over C_{30} from Integration Report.

Total Area = 7.85E8
ClC_8 Area = 1.19E5
ClC_{18} Area = 1.48E5
$< C_7$ Area = 7.84E8
$> C_{30}$ Area = 1.37E4

2) Calculate DRO Area

DRO Area = Total Area - (ClC_8 + ClC_{18} + $< C_7$ + $> C_{30}$)
= 7.85E8 - (1.19E5 + 1.48E5 + 7.84E8 + 1.37E4)
= 3.68E5

3) Calculate ClC_8, ClC_{18} and DRO concentrations from Standards RF.

ClC_8 Conc (mg/L) = ClC_8 Area / RF ClC_8
ClC_{18} Conc (mg/L = ClC_{18} Area / RF ClC_{18}
DRO Conc (mg/L) = 167.5274 + (1.0065E-3 * DRO Area)
(from July 1995, Run #1 Calibration Curve)

ClC_8 Conc = 1.19E5 / 2352.725 $(mg/L)^{-1}$
= 50.23 mg/L

ClC_{18} Conc = 1.48E5 / 2522.598 $(mg/L)^{-1}$

= 58.54 mg/L

DRO Conc = 167.5274 + (1.0065E-3 * 3.68E5)

= 537.44 mg/L

4) Calculate % Recovery for ClC_8 and ClC_{18}.

% ClC_8 Recovery = {ClC_8 Conc / [(4.375 * 0.1) / V]} * 100

% ClC_{18} Recovery = {ClC_{18} Conc / [(4.245* 0.1) / V]} * 100

where,

ClC_8 Conc: from step 3) (mg/L)

ClC_{18} Conc: from step 3) (mg/L)

4.375: ClC_8 concentration in recovery solution (mg/mL)

4.245: ClC_{18} concentration in recovery solution (mg/mL)

0.1: amount of recovery solution added to sample (mL)

V: sample extract volume (L)

% ClC_8 Recovery = {50.23 / [(4.375 * 0.1) / 0.008]} * 100

= 91.85 %

% ClC_{18} Recovery = {58.54 / [(4.245 * 0.1) / 0.008]} * 100

= 110.32 %

Note: for samples where % ClC_8 and ClC_{18} Recoveries were assessed as problematic, Corrected DRO concentrations were calculated as follows:

Corrected

DRO Conc(mg/L) = DRO Conc + {DRO Conc * [(100-% ClC_{18} Recovery)/100]}

where,

DRO Conc: from step 3) (mg/L)

This Corrected DRO concentration would replace DRO Conc in the following steps.

5) Calculate sample T.E.H. concentration per dry weight of soil.

T.E.H. (mg/kg) = (DRO Conc * V) / {[Mww - (SMC * Mww)] / 1000}

where,

DRO Conc: from step 3) (mg/L)

Mww: wet weight of soil in extraction timble (g)

SMC: soil moisture content (fraction)

1000: g to kg conversion factor

T.E.H. = (537.44 * 0.008) / {[10.3716 - (0.2695 * 10.3716)] / 1000}

= 567.49 mg/kg

Integration Report

HEWLETT-PACKARD PART NUMBER 5181-1219 PI

RUN# 99 AUG 2, 1995 21:48:27

ED-C-01-950729-1B

SAMPLE NAME: SAMPLE# 2

METHOD NAME: M:TPH.MET

VOC ANALYSIS

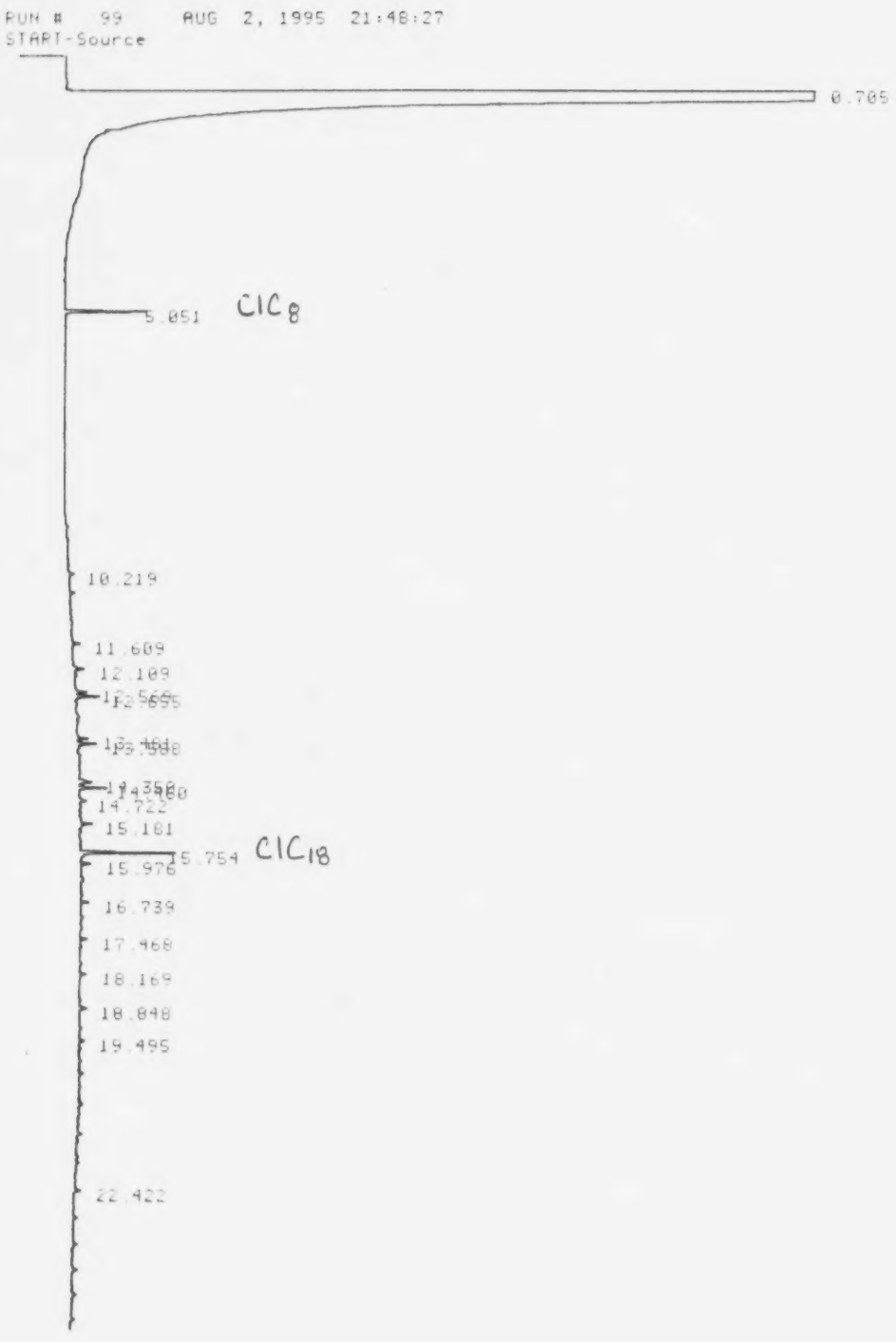
AREA%

	RT	AREA	TYPE	WIDTH	AREA%
	.705	784382400	SHB	.050	99.91686
C1C6 -	5.051	118682	PB	.036	.01512
	10.219	10259	BB	.041	.00131
	11.609	16366	PB	.044	.00208
	12.109	13942	BB	.033	.00178
	12.569	29827	BV	.054	.00380
	12.655	47147	VP	.045	.00601
	13.481	16650	BP	.036	.00212
	13.588	34637	PV	.042	.00441
	14.350	32411	PV	.059	.00413
	14.460	41121	VB	.036	.00524
	14.722	14057	PB	.048	.00179
	15.181	28716	VV	.052	.00366
C7C18 -	15.754	147663	PV	.037	.01881
	15.976	17976	BV	.039	.00229
	16.739	12953	PB	.035	.00165
	17.468	10023	PB	.031	.00128
	18.169	10312	PB	.035	.00131
	18.848	21309	PV	.056	.00271
	19.495	15802	VP	.055	.00201
	22.422	13730	BP	.046	.00175

TOTAL AREA=7.8503E+08

MUL FACTOR=1.0000E+00

GC - Fingerprint



APPENDIX E - Molar C:N:P Calculations

A sample calculation is provided based on Cell A, May 1995 results.

1) Calculate molar C content based on mean T.E.H. level in Cell.

C_a (mol/kg) = mean T.E.H. * (1/1000) * (1/12)

where
mean T.E.H.: mean T.E.H. level in Cell (mg/kg)
1000: mg to g conversion factor
12: molar weight of carbon (g/mol)

C_a = 2204.333 * (1/1000) * (1/12)
= 0.1837 (mol/kg)

2) Calculate available molar N content based on ammonia-N and nitrate-N analyses results.

N_a (mol/kg) = [NH_3-N * (1/1000) * (1/14)] + [NO_3-N * (1/1000) * (1/14)]

where
NH_3-N: ammonia-N analysis result (mg/kg)
NO_3-N: nitrate-N analysis result (mg/kg)
1000: mg to g conversion factor
14: molar weight of nitrogen (g/mol)

N_a = [2.6 * (1/1000) * (1/14)] + [9 * (1/1000) * (1/14)]
= 0.0008 mol/kg

3) Calculate available molar P content based on PO_4-P analysis results.

P_a (mol/kg) = [PO_4-P * (1/1000) * (1/31)]

where

PO_4-P: orthophosphate-P analysis result (mg/kg)

1000: mg to g conversion factor

31: molar weight of phosphorus (g/mol)

P_a = [5.9 * (1/1000) * (1/31)]

= 0.0002 mol/kg

4) Calculate existing molar C : N : P.

C = 100

N = N_a / C_a * 100

P = P_a / C_a * 100

where all terms have been defined above

C = 100

N = 0.0008 / 0.1837

= 0.45

P = 0.0002 / 0.1837

= 0.10

C : N : P = 100 : 0.5 : 0.1

APPENDIX F - Detailed Analysis of 1994 and 1995 Soil Conditions and Treatment Operations for Edmonton RBF

Soil Conditions

Metals

Results from 1994 and 1995 metals analyses are included in Table F1. The COV for each metal analysis was calculated for each year in order to assess the variation among each cell. Metals for which the COV were higher than 20% were investigated for possible effects on the bioremediation process. As the analytical method used (ICP-Atomic Emission spectroscopy) did not give any indication on speciation or oxidation state of metals, it is difficult to compare metal concentrations to expected ranges; where possible comments were made on the possible effects of metal concentrations.

In 1994, the cells had significant variations in levels of Na, Ti, Zr and As. For all these metals except Zr, Cell A value was the median value and therefore these differences are not thought to be the cause for the poor T.E.H. removals obtained in Cell A. The level of Zr was lower in Cell A; as Zr is not thought to be a requirement for microbial activity it was concluded that the low level would not impede the bioremediation process. In 1995, the cells had a significant difference in levels of Ca, Ni and Na; the differences in Ca and Na will be discussed below. Ni was not present in Cell A in May 1995 (MDL: 2 mg/kg); this result is surprising as all cells had comparable Ni levels in May 1994. Ni is a minor bioelement but has not been identified as an essential nutrient for most microorganisms (Alexander, 1977).

There are some trends between 1994 and 1995 results. The presence of Pb and Se in 1995 can be explained by a reduction in MDL from 10.0 to 5.0 mg/kg and from 0.5 to 0.1 mg/kg for Pb and Se respectively. In general there was an increase in most analytical results; this might be explained by the use of a microwave digestion in 1995 vs conventional heating on a hot plate in 1994. However Ca, Mg and Na levels reported for 1995 are considerably lower than those for 1994; in 1994 ICP was used on an acid digestion of the soil sample whereas in 1995 it was used on the saturated paste extract (water extraction).

Table F1. 1994 and 1995 Metals Analyses Results for Edmonton RBF.

(mg/kg)

Metal	Cell A		Cell C			Cell D		
	May 1994	May 1995	May 1994	May 1995	Oct 1995	May 1994	May 1995	Oct 1995
Al	5160.0		5470.0			5690.0		
Ag	< 1.0	< 1	< 1.0	< 1	(< 1)	< 1.0	< 1	(< 1)
As	5.90	5.2	8.10	4.4	(2.7)	4.70	4.8	(2.2)
Ba	118.00	175	121.00	177	(230)	117.00	173	(230)
Be	< 0.5	< 1	< 0.5	< 1	(< 1)	< 0.5	< 1	(< 1)
Bo	44.80		53.30			48.40		
Ca	9330	449.58	6640	110.88	(119)	7040	257.4	(99)
Cd	< 1.0	< 0.5	< 1.0	< 0.5	(<0.5)	< 1.0	< 0.5	(<0.5)
Co	7.10	7	7.40	7	(8)	7.00	7	(7)
Cr	8.20	23.1	8.70	26.0	(57.9)	8.80	20.0	(43.7)
Cu	16.40	22	17.60	23	(28)	17.10	23	(24)
Fe	12800		15500			13800		
Hg	0.027	0.02	0.028	0.02	(0.04)	0.022	0.02	(0.04)
Mg	3380	79.06	3030	26.64	(44.5)	3100	48.6	(32.4)
Mn	260.00		310.00			280.00		
Mo	< 2.0	< 1	< 2.0	< 1	(< 1)	< 2.0	< 1	(< 1)
Na	330.00	43.66	200.00	37.44	(32)	420.00	20.4	(24)
Ni	18.80	< 2	20.10	26	(65)	21.00	24	(40)
P	320.00	539	390.00	531		310.00	549	
Pb	< 10.0	11	< 10.0	12	(29)	< 10.0	10	(17)
Sb	< 1.0		< 0.5			< 1.0		
Se	< 0.5	0.3	< 0.5	0.3	(0.5)	< 0.5	0.2	(0.3)
Sn		< 5		< 5	(7)		< 5	(6)

( ): indicate new soils

Table F1. 1994 and 1995 Metals Analyses Results for Edmonton RBF - Continued.

(mg/kg)

Sr	28.10	44	30.90	43	(60)	30.40	42	(57)
Ti	13.7		24.1			11.7		
Tl		< 1		< 1	(< 1)		< 1	(< 1)
Va	16.80	27	18.60	28	(36)	17.90	25	(29)
Zn	73.30	66.8	72.80	76.5	(160)	87.80	68.2	(90.9)
Zr	4.40		6.50			4.70		

( ): indicate new soils

EC

EC results for 1994 and 1995 are included in Table F2. The EC of a soil determines the total solute concentration in a soil extract. It reflects the content of soluble salts in a soil which can have detrimental effects on biological activity. Although conductivity levels are usually assessed against crop growth it can also have adverse effects on soil structure. All 1994 results are within the range where the salinity would have almost negligible effects on crop growth (CSSS, 1993). There was an apparent increase in conductivity in all cells in 1995 but all cells are still within a comparable range; for 1995 the reported conductivities would restrict yields of very sensitive crops (CSSS, 1993). It is to note that the CCME Criteria for Commercial/Industrial remediation is 4 dS/m (4000 μS/cm).

Table F2. 1994 and 1995 EC Analyses Results.

(μS/cm)

Sampling Event	Cell A	Cell C	Cell D
May 94	430.00	430.00	350.00
July 94	420.00	330.00	340.00
Oct 94	810.00	710.00	830.00
May 95	3900	1200	2600
July 95	2900	3600	4300
Oct 95	3950	(1870)	(1720)

( ): indicate new soils

The extracts used to measure the conductivity were different in 1994 and 1995. In 1994, the conductivity was measured on a 1:2 soil:water ratio mixture and in 1995 the conductivity was measured on a saturated soil paste extract. The technique offering most accurate characterization of ionic conditions in the soil environment is the direct extraction of the soil solution. Soil solution extraction, however, is possible only under relatively high soil moisture conditions and is time consuming. The most common method of extraction, used almost universally in the analysis of soil salinity, is the saturation paste extraction (CSSS, 1993). The 1:2 soil:water ratio mixture would result in greater dilution of salts which could explain the lower conductivity.

The predominant solutes responsible for salinity include the cations sodium, calcium and magnesium and the anions sulfate and chloride; minor amounts of potassium, bicarbonate, carbonate, and nitrate may also be present. There should exist a correlation between the levels of solutes and the conductivity. Detailed salinity analyses were not conducted in 1994; as salinity analyses are conducted on aqueous extracts of soils, the Ca, Mg and Na analyses which were performed on an acid digestion of the soil samples in 1994 can not be related to soil salinity. To provide a correlation for the 1995 conductivity analyses, 1995 detailed salinity analyses results are reported in Table F3.

Table F3. 1994 and 1995 Detailed Salinity Results.

(mg/L)

Ion	Cell A			Cell C			Cell D		
	May	July	Oct	May	July	Oct	May	July	Oct
Calcium	762	489	637	154	535	(206)	429	725	(198)
Chloride	55	49	43.9	42	46	(57.1)	36	38	(55.1)
Potassium	22	26	22.9	11	24	(14.3)	24	30	(15.3)
Magnesium	134	102	133	37	124	(76.7)	81	144	(64.8)
Sodium	74	80	107	52	64	(56)	34	56	(47)
Sulfate	1520	411	900	119	162	(399)	392	418	(332)

( ): indicate new soils

With the exception of chloride, all ions listed in Table F3 are required for microbial metabolism (Riser-Roberts, 1994). Minimum calcium concentrations of 200 mg/L have been reported as being sufficient (Riser-Roberts, 1994). Although particular levels for the other ions were not verified, these results show that Cell A conditions are similar to Cells C and D.

SAR

Results for 1994 and 1995 SAR are reported in Table F4. The SAR is a useful index of the sodicity or relative sodium status of soil solutions, aqueous extracts, or water in equilibrium with soil. It is calculated as

$$SAR = [Na^+]/[Ca^{2+} + Mg^{2+}]^{0.5} \qquad (F1)$$

As Na^+ ions are highly hydrated, loosely held monovalent ions, an overabundance can cause dispersion which in turn will lead to lower permeability and swelling.

Results for all three cells are within the same ranges when considered on a yearly basis. The difference between 1994 and 1995 results can be explained by the fact that the results for 1994 are based on a MDL of 1.0.

Table F4. 1994 and 1995 SAR Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 94	0	0	0
Jul 94	0	0	0
Oct 94	0	0	0
May 95	0.7	1.0	0.4
Jul 95	0.9	0.6	0.5
Oct 95	1.0	(0.8)	(0.7)

( ): indicate new soils

TOC

Results for 1994 and 1995 TOC analyses are reported in Table F5. Increases in TOC levels in 1994 are due to the different analytical methods used in May (wet combustion-titration method) and July and October (gravimetric procedure). The method used in 1995 was a wet oxidation-redox titration method and the values reported are for organic carbon. May 1994 and 1995 values are comparable. TOC contents were consistently in the same ranges for all three cells.

Table F5. 1994 and 1995 TOC Analyses Results.

(%)

Sampling Event	Cell A	Cell C	Cell D
May 94	2.09	2.35	1.48
July 94	7.30	6.70	6.30
Oct 94	9.40	6.70	5.80
May 95	2.3	2.1	2.3
July 95	3.3	2.7	3.6
Oct 95	2.7	(2.2)	(1.8)

( ): indicate new soils

pH

Results for 1994 and 1995 pH analyses are reported in Table F6. All values are within or slightly below the optimal range for bioremediation of 7 to 8. (Bossert and Bartha, 1984).

Table F6. 1994 and 1995 pH Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 94	7.40	7.40	7.40
July 94	7.30	7.40	7.10
Oct 94	6.80	6.80	6.70
May 95	7.3	7.4	7.4
July 95	7.2	7.3	7.2
Oct 95	7.8	(7.8)	(7.7)

( ): indicate new soils

Nutrients

Results for ammonium and nitrate for 1994 and 1995 are presented in Table F7 and F8 respectively. The high ammonia-N levels and absence of nitrate-N in May 1994 could be an indication of anaerobic soil conditions as in the absence of oxygen, nitrate

may be used by microorganisms as an electron acceptor and become reduced to NH_4^+ (Paul and Clark, 1994). Although ammonia-N concentrations were similar, nitrate-N concentrations appeared to be lower in Cell A in both Oct 1994 and July 1995. This observation will be discussed further below.

Table F7. 1994 and 1995 Ammonium Analyses Results.

(mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 94	62.0	56.0	82.0
July 94	0.0	0.0	0.0
Oct 94	58.0	41.0	63.0
May 95	2.6	1.6	6.8
July 95	98	182	95
Sep 95		(0.6)	(0.8)
Oct 95	38.8	(39.6)	(18.6)

( ): indicate new soils

Table F8. 1994 and 1995 Nitrate Analyses Results.

(mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 94	0.0	0.0	0.0
July 94	0.0	0.0	0.0
Oct 94	85.6	125.0	197.0
May 95	9.0	5.4	13.0
July 95	172	252	280
Sep 95		(34.2)	(30.6)
Oct 95	240	(94.0)	(62.0)

( ): indicate new soils

In order to assess any differences in nutrient status between cells the molar C:N ratios were calculated based on the mean T.E.H. levels at the time of sampling and results are presented in Table F9. Results for 1994 were calculated using the same methodology as for 1995 results.

Table F9. 1994 and 1995 Molar C : N Ratio Calculation Results.

Sampling Event	Cell A	Cell C	Cell D
May 94	100 : 0.7	100 : 0.6	100 : 1.2
July 94	100 : 0.0	100 : 0.0	100 : 0.0
Oct 94	100 : 1.4	100 : 1.9	100 : 3.8
May 95	100 : 0.5	100 : 0.2	100 : 1.0
July 95	100 : 11	100 : 43	100 : 47
Sep 95		(100: 1.2)	(100: 2.3)
Oct 95	100 : 40	(100: 10)	(100: 12)

( ): indicate new soils

When compared within sampling events all cells are constantly in the same ranges i.e. either well below or well above the target ratio of 100 : 5.

Although total P was not used in the calculation of available nutrients in 1995, total P analysis was conducted as part of the ICP metals analyses in May 1995 and results are compared to 1994 results in Table F10. Differences in extration method, as discussed above explains the apparent increase in total P between 1994 and 1995. In 1995 calculation of available phosphorus was based on orthophosphates levels which are reported in Table F11.

Table F10. 1994 and 1995 Total Phosphorus Analyses Results.

(mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 94	340.0	250.0	400.0
July 94	94.0	240.0	230.0
Oct 94	250.0	310.0	280.0
May 95	539	531	549

Table F11. 1995 Orthophosphate Analyses Results.

(mg/kg)

Sampling Event	Cell A	Cell C	Cell D
May 95	5.9	13.2	12.1
July 95	28.0	37.5	75.0
Sep 95		(3.1)	(2.4)
Oct 95	38.0	(9.3)	(5.8)

( ): indicate new soils

In order to better assess the nutrient status of the cells, the molar C:P ratios were calculated based on orthophosphate levels and mean T.E.H. levels at the time of sampling and are reported in Table F12. Note that although total P is not representative of P availability to microorganisms, as the amount of soil phosphorus available for uptake by living organisms is considered to be the portion that is extractable by dilute acid or bicarbonate, 1994 C : P ratios are included only as a means of comparing between-cell results.

Table F12. 1994 and 1995 Molar C : P Ratio Calculation Results.

Sampling Event	Cell A	Cell C	Cell D
May 94	100 : 1.6	100 : 1.2	100 : 2.7
July 94	100 : 0.8	100 : 2.2	100 : 1.5
Oct 94	100 : 1.1	100 : 1.6	100 : 1.8
May 95	100 : 0.1	100 : 0.2	100 : 0.3
July 95	100 : 0.5	100 : 1.7	100 : 4.3
Sep 95		(100 : 0.04)	(100 : 0.08)
Oct 95	100 : 2.4	(100 : 0.3)	(100 : 0.4)

( ): indicate new soils

Cells A and D follow similar trends between May and October 1994. Results for 1995 can be compared against the target ratio of 100 : 1. Similar conditions prevailed in all cells in May 1995. July 1995 results show a deficiency in phosphoruus in Cell A but excesses in Cells C and D. The excesses in Cells C and D can be partly due to the low T.E.H. levels from which the C:P ratios were calculated. However Table F11 indicates higher orthophosphate concentrations in Cell C and D in July 1995. This observation will be further discussed below when reviewing RBF operations.

Moisture Content

Results for moisture content analyses for 1994 and 1995 are reported in Table F13. Except for the higher moisture levels in June 1994, moisture content levels were within or very close to being within the recommended range of 15 to 20 % (Frankenberger, 1992).

Table F13. 1994 and 1995 Moisture Content Analyses Results.

(% w/w dry soil)

Sampling Event	Cell A	Cell C	Cell D
May 94	14.80	15.70	12.30
June 94	23.00	26.00	24.00
July 94	20.00	22.00	20.00
Aug 94	21.00	19.00	16.00
Oct 94	15.00	15.00	14.00
May 95	17	17	15
July 95	21	22	22
Sep 95		(19)	(18)
Oct 95	25	(21)	(21)

( ): indicate new soils

Particle Size Analysis

1994 and 1995 results for particle size analysis are reported in Table F14. Results from May 1995 indicate soils in all three cells have similar textures and can be considered as clay (Pierzynski, 1994).

Table F14. 1994 and 1995 Particle Size Analyses Results.

Sampling Event	Cell A	Cell C	Cell D
May 94	30% sand	30% sand	34% sand
	68% silt or clay	69% silt or clay	62% silt or clay
May 95	37.6% sand	36.6% sand	35.6% sand
	21.0 % silt	20.0 % silt	22.0 % silt
	42.4 % clay	44.4 % clay	40.4 % clay
Oct 95		(42.9 % sand)	(44.1 % sand)
		(21.3 % silt)	(20.0 % silt)
		(35.9 % clay)	(35.9 % clay)

( ): indicate new soils

Microtox®

Results for Microtox® analyses for 1994 and 1995 are reported in Table F15. As the results indicate the concentration in percentage required to reduce the luminescence of the bacteria by half, a lower percentage is associated with a higher toxicity. June 1994 results indicate the following ranking in toxicity: Cell C < Cell A < Cell D. By October 1994 all cells appeared to have lost their toxicity. Results from analyses performed on the original soils in Cells A, C and D indicate all WSF to have very low toxicity.

Table F15. 1994 and 1995 Microtox® Analyses Results (EC50, 5 min).

(%)

Sampling Event	Cell A	Cell C	Cell D
June 94	20	82	4.1
Oct 94	>100	>100	>100
May 95	>100	>100	>100
July 95	>100	>100	>100
Oct 95	> 100	(29 - > 100)	(> 100)

( ): indicate new soils

It is interesting to note that although the toxicity of the WSF was reduced between June and October 1994, the T.E.H. levels remained practically unchanged from May to October. This can be explained by the fact that toxicity is usually associated with the short-chain paraffins below nC_{10} which are usually removed through volatilization.

It has been reported that biodegradation products generally also increase the acute toxicity of hydrocarbon pollutants (Wang and Bartha, 1994). This is due in part to the direct effect of metabolic intermediates such as fatty acids and phenols and indirectly, to their contribution increase dispersion of the remaining intact hydrocarbons by their detergent action. This increase in toxicity is usually temporary. The opportunity

to observed such an increase was most likely restricted by the testing frequency in 1994. Detoxification occured and 1995 results showed that it appears to be permanent.

Microbial Enumeration

Microbial enumeration results are reported in Table F16. For 1995 each results represent the mean and standard error of three replicates, however such details were not provided by the commercial laboratory where microbial enumeration analyses were performed in 1994. For 1994, results represent the levels of HUB i.e. only those strains capable of utilizing diesel as sole carbon source. A standard plate count method was used to obtain these counts except that a diesel fuel was used as the sole carbon source in the growth medium. These analyses were performed in a commercial laboratory and, unfortunately, details with respect to the type of growth medium and type of diesel fuel were not provided. Frequently, anywhere from < 1% to 10% of the total bacteria in soil consist of hydrocarbon oxidizers (Frankenberger, 1992). For 1995, results are the total heterotrophic counts using standard plate count agar. It is known that this agar yields lower counts than other agars used in the Plate Count technique (APHA, 1994). May and July 1995 results for all cells are within the range associated with fertile soils, 10^7 to 10^8 CFU/g dry soil (Riser-Roberts, 1994).

Table F16. 1994 and 1995 Microbial Enumeration Results.

(CFU/g soil)

Sampling Event	Cell A	Cell C	Cell D
Oct 94 (HUB)	1.0×10^6	1.2×10^6	2.0×10^5
May 95 (THB)	1.12E7 ± 0.06E7	7.67E6 ± 0.16E6	6.06E6 ± 0.10E6
July 95 (THB)	1.30E7 ± 0.02E7	8.72E6 ± 0.32E6	8.57E6 ± 1.23E6
Oct 95 (THB)	8.60E5 ± 0.12E5	(6.65E4 ± 0.34E4)	(6.60E5 ± 0.49E5)

( ): indicate new soils

Changes in microbial numbers are often observed throughout the biodegradation process. Levels as high as 10^{11} bacteria/g soil for THB and 10^{10} bacteria/g soil for HUB have been reported (Chaineau et al., 1995). This changes have been shown to occur within weeks of hydrocarbon contamination with levels returning to normal within six to eight weeks (Wang and Bartha, 1994). However, such changes were not observed within the 1995 monitoring framework.

When considering May and July 1995 results, there is a significant difference between the microbial count in Cell A vs the microbial counts in Cell C and D (t-test, $\alpha = 0.05$). The higher microbial count in Cell A does not seem to be related to T.E.H. levels as the three cells had similar levels in May 1995, refer to Table 7, Section 3.4.3. However they could explain some of the lower nutrient concentration observed in Cell A as nutrient requirements in that cell would be greater.

Microbial enumeration results for 1994 and 1995 are not readily comparable. It has been reported that the bacterial population density required for successful biodegradation of petroleum products is greater than 10^6 CFU/g soil (Fan and Tafuri, 1994) and May and July 1995 results indicate that soils from all three cells contained populations that could support the biodegradation process.

Treatment Operations

Treatment operations at Edmonton RBF for 1994 and 1995 are summarized in Tables F17 and F18 respectively. All cells were treated similarly throughout 1994 and up until Aug in 1995 when soils in Cells C and D were removed. A major difference between 1994 and 1995 was the frequency of nutrient addition and tilling. It was noted in the facility logbook that wet soil conditions prevailed throughout June and July 1994 due to weather conditions and drainage problems within the cells and as a result access to the cells was restricted (CH2M Hill, 1994). The cells were only tilled three times in 1994 whereas they were tilled almost weekly throughout May and June 1995. Nutrients were were not added until Aug in 1994 whereas they were added monthly starting in June in 1995.

As the amount of inorganic nutrients added in all cells was similar, differences in resulting concentrations could be due to differences in soil densities and cell dimensions as these were all assumed to be similar. In fact, measurement of the cells dimensions in Sep 1995 revealed that Cell A was somewhat bigger resulting in an increase in soil mass by a factor of 1.2.

Table F17. Summary of 1994 Edmonton RBF Operation.

Date	Fertilizer Application			Irrigation			Tillage		
	Cell A	Cell C	Cell D	Cell A	Cell C	Cell D	Cell A	Cell C	Cell D
May 30							X	X	X
July 27				system test					
Aug 24							X	X	X
Aug 31	265 kg N 100 kg P	265 kg N 100 kg P	265 kg N 100 kg P						
Sep 19	305 kg N 120 kg P	305 kg N 120 kg P	305 kg N 120 kg P				X	X	X

X: event

Table F18. Summary of 1995 Edmonton RBF Operation.

Date	Fertilizer Application			Irrigation			Tillage		
	Cell A	Cell C	Cell D	Cell A	Cell C	Cell D	Cell A	Cell C	Cell D
May 1							X	X	X
May 8							X	X	X
May 15							X	X	X
May 23							X	X	X
May 30							X	X	X
Jun 6							X	X	X
Jun 13	86.25 kg N	86.25 kg N	86.25 kg N	system test	system test	system test	X	X	X
Jun 26	124.5 kg N 89.25 kg P	162 kg N 102 kg P	98.25 kg N 51 kg P				X	X	X
July 17							X	X	X
July 20	124.5 kg N 89.25 kg P	162 kg N 102 kg P	93 kg N 102 kg P						

X: event

Table F18. Summary of 1995 Edmonton RBF Operation Continued

Aug 21							X	X	X
Aug 23	124.5 kg N 89.25 kg P								
Aug 28							X	X	X
Oct 12	144 kg N 64 kg P	144 kg N 64 kg P	144 kg N 64 kg P	X	X	X			
Oct 20	61 kg N	61 kg N	61 kg N				X	X	X

X: event

APPENDIX G - Factorial Design Analysis

1) Reductions in T.E.H. concentrations were calculated for each condition.

Reduction = T.E.H. Wk 0 - T.E.H. Wk 8

2) For each condition, Mean Reduction and Variance were calculated based on three Reduction results. Excel 7.0 statistical analyses were used to calculate means and variances. Results are presented in Table G1.

3) Effects and interaction were calculated using the Table of Signs method. Based on Table G2,

NA = (- 3395.29 + 4169.01 - 3490.17 + 4151.11) / 2

AS = (3395.29 + 4169.01 - 3490.17 - 4151.11) / 2

NA-AS = (-3395.29 + 4169.01 + 3490.17 - 4151.11) / 2

Results are presented in Table G3.

4) The standard error of the effects was calculated using methodology described by Box et al. (1978). For each condition, the three samples were considered as replicates. These replicates were obtained under similar experimental conditions (i.e. from the same beaker), but because of the heterogeneous nature of the system it was considered that the three samples would adequately provide a reflection of total variability.

St Error (effect) = $\sqrt{}$ V(effect)

where

V (effect) = [4 * (Pooled Var)] / N_t

where

N_t = # of samples used in the analysis = 12, and

Pooled Var = $(\nu_1 s_1^2 + \nu_2 s_2^2 + ... + \nu_4 s_4^2) / \nu_1 + \nu_2 + ... + \nu_4$

where for each 4 conditions, s_i^2 is the estimate of variance on T.E.H. reduction and $\nu_i = n_i - 1$ degrees of freedom associated with the $n_i = 3$ replicates. Results of these calculations are presented in Table G4.

5) When comparing the effects with their standard error, it can be seen that while AS and N-AS could have been generated by noise, addition of nutrient (NA) yielded significantly different results. The positive sign of NA indicates that addition of nutrient produced an increased mean reduction in T.E.H. levels.

Table G1. Statistical Analysis.

	T.E.H. Wk 0 (mg/kg)	T.E.H. Wk 8 (mg/kg)	Reduction (mg/kg)	Mean Reduction (mg/kg)	Variance ((mg/kg)^2)
ED-2-A-	4684.81	486.66	4198.15	4169.01	818.30
ED-2-B-	4684.81	543.84	4140.97		
ED-2-C-	4684.81	516.90	4167.91		
ED-4-A-	4684.81	487.50	4197.31	4151.11	35916.31
ED-4-B-	4684.81	742.04	3942.77		
ED-4-C-	4684.81	371.55	4313.26		
ED-1-A-	4684.81	1337.41	3347.40	3395.29	11643.24
ED-1-B-	4684.81	1365.19	3319.62		
ED-1-C-	4684.81	1165.96	3518.85		
ED-3-A-	4684.81	1090.19	3594.62	3490.17	12621.39
ED-3-B-	4684.81	1180.23	3504.58		
ED-3-C-	4684.81	1313.49	3371.32		

Table G2. Table of Signs.

Condition #	NA	AS	NA-AS	Mean Reduction (mg/kg)
1	-1	1	-1	3395.29
2	1	1	1	4169.01
3	-1	-1	1	3490.17
4	1	-1	-1	4151.11

Table G3. Table of Signs Calculation Results.

Effects:	
NA:	717.33
AS:	-38.49
NA-AS:	56.39

Table G4. Variance and Standard Error of Effects.

V(effect):	5083.27
st error (effect):	71.30

APPENDIX H - Regression Analysis for First-Order Modeling for Bench-Scale Experiment

Summary outputs, line fit plots, and residual plots from the regression analysis are presented for Conditions # 1 to # 4. A Table summarizing Sum of Squares calculations is also included. Microsoft® Excel 7.0 statistical analysis package was used for all statistics.

SUMMARY OUTPUT - Condition # 1

Regression Statistics	
Multiple R	0.76881898
R Square	0.59108263
Adjusted R Square	0.56956066
Standard Error	0.37595105
Observations	21

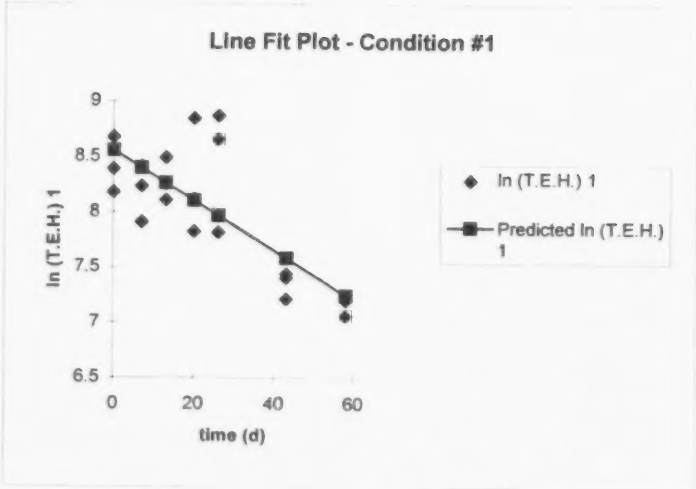
Lack of Fit

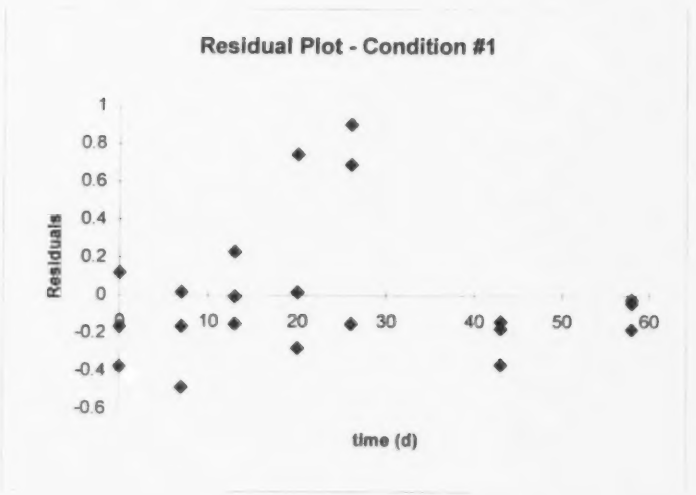
	SS	df	MS	F	P
Residual	2.6854	19			
Error	1.547	14	0.1105	2.06	0.13
Lack of Fit	1.1384	5	0.2277		

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.881761425	3.881761	27.46415	4.65793E-05
Residual	19	2.685444659	0.141339		
Total	20	6.567206083			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	8.55465868	0.131754583	64.92874	8.93E-24	8.278893088	8.830424
time (d)	-0.0226468	0.004321399	-5.24063	4.66E-05	-0.031691627	-0.0136


Line Fit Plot - Condition #1
ln (T.E.H.) 1
time (d)
ln (T.E.H.) 1
Predicted ln (T.E.H.) 1
9
8.5
8
7.5
7
6.5
0
20
40
60


Residual Plot - Condition #1
Residuals
time (d)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
10
20
30
40
50
60

SUMMARY OUTPUT - Condition # 2

Regression Statistics	
Multiple R	0.92419531
R Square	0.85413697
Adjusted R Square	0.84645997
Standard Error	0.2989408
Observations	21

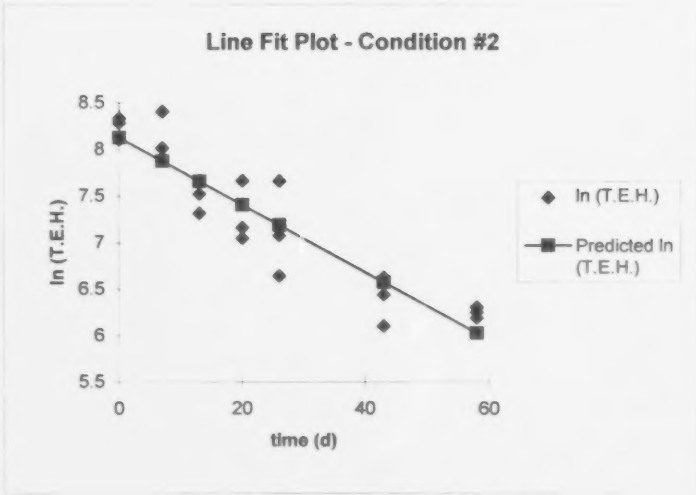
Lack of Fit

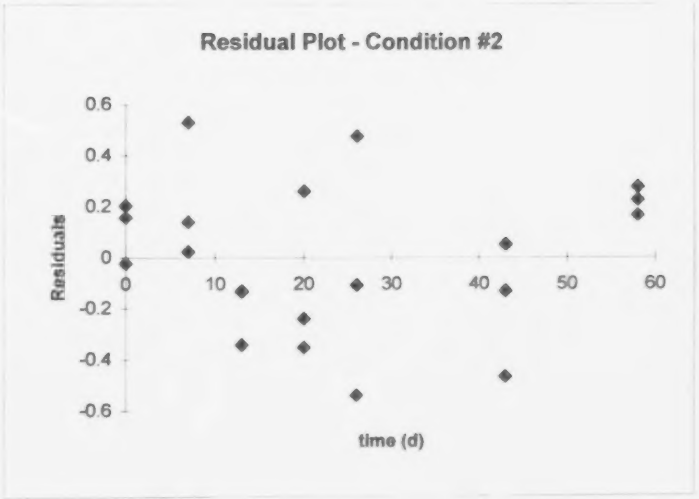
	SS	df	MS	F	P
Residual	1.6979	19			
Error	1.0803	14	0.0772	1.6	0.22
Lack of Fit	0.6176	5	0.1235		

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	9.942743966	9.942744	111.2592	2.21359E-09
Residual	19	1.697946385	0.089366		
Total	20	11.64069035			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	8.12604232	0.104765819	77.56387	3.08E-25	7.906764869	8.34532
time (d)	-0.0362448	0.003436198	-10.5479	2.21E-09	-0.04343689	-0.029053


Line Fit Plot - Condition #2
ln (T.E.H.)
time (d)
8.5
8
7.5
7
6.5
6
5.5
0
20
40
60
ln (T.E.H.)
Predicted ln (T.E.H.)


Residual Plot - Condition #2
Residuals
time (d)
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0
10
20
30
40
50
60

SUMMARY OUTPUT - Condition # 3

Regression Statistics	
Multiple R	0.8144048
R Square	0.6632552
Adjusted R Square	0.6455318
Standard Error	0.3752484
Observations	21

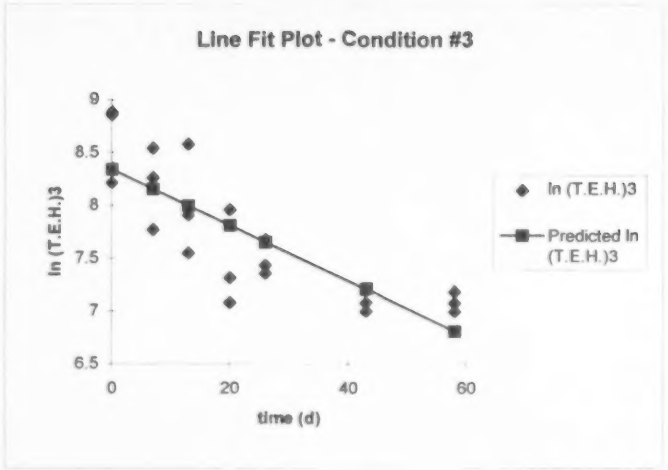
Lack of Fit

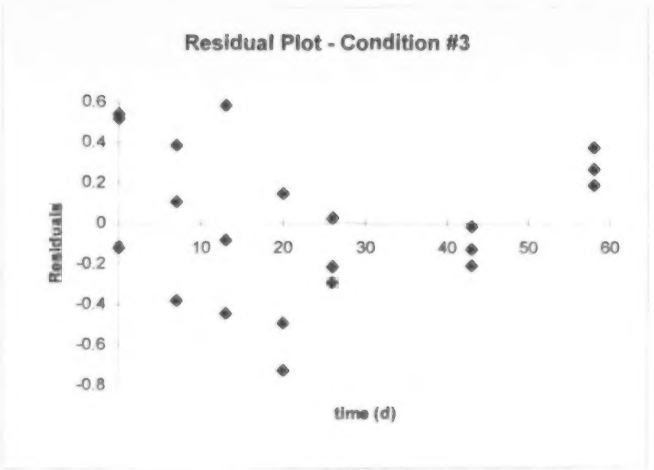
	SS	df	MS	F	P
Residual	2.6754	19			
Error	1.6369	14	0.1169	1.78	0.18
Lack of Fit	1.0385	5	0.2077		

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5.269521428	5.269521	37.42255	7.00469E-06
Residual	19	2.675416491	0.140811		
Total	20	7.944937919			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	8.3357513	0.131508349	63.38572	1.41E-23	8.060501086	8.611002
time (d)	-0.0263863	0.004313323	-6.1174	7E-06	-0.0354142	-0.01736


Line Fit Plot - Condition #3
ln (T.E.H.)3
time (d)
ln (T.E.H.)3
Predicted ln (T.E.H.)3


Residual Plot - Condition #3
Residuals
time (d)

SUMMARY OUTPUT - Condition # 4

Regression Statistics	
Multiple R	0.9307274
R Square	0.8662536
Adjusted R Square	0.8578944
Standard Error	0.3040208
Observations	18

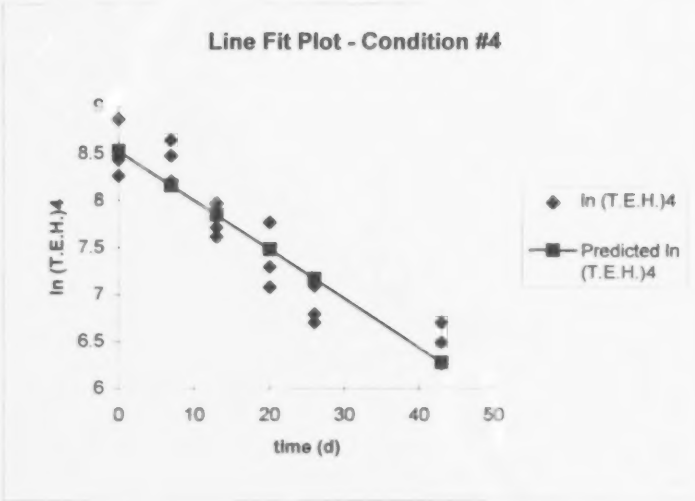
Lack of Fit

	SS	df	MS	F	P
Residual	1.4789	16			
Error	0.7838	12	0.0653	2.66	0.08
Lack of Fit	0.6951	4	0.1738		

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	9.578319661	9.57832	103.6294	2.14228E-08
Residual	16	1.478858065	0.092429		
Total	17	11.05717773			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	8.5236397	0.117809539	72.35101	1.46E-21	8.273894648	8.773385
time (d)	-0.0523993	0.005147352	-10.1799	2.14E-08	-0.063311175	-0.04149


Line Fit Plot - Condition #4
ln (T.E.H.)4
time (d)
ln (T.E.H.)4
Predicted ln (T.E.H.)4

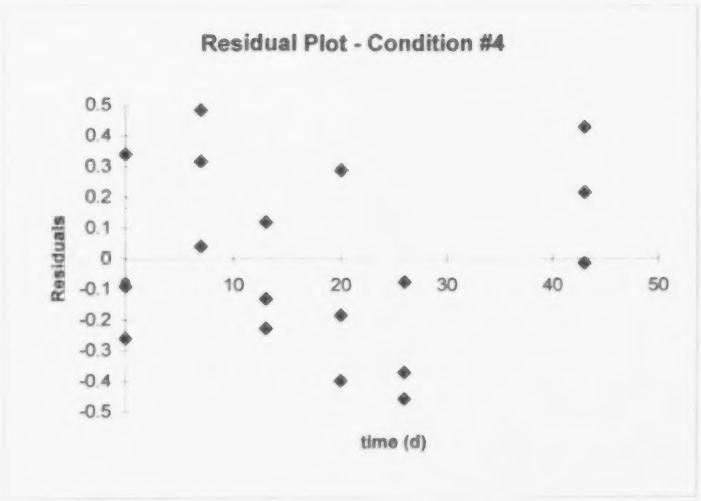

Residual Plot - Condition #4
Residuals
time (d)

Table G1. Error Sum of Squares.

	ln DATA				Sum of Squares of Deviations			
time (d)	#2	#4	#1	#3	#2	#4	#1	#3
0	8.1017	8.8631	8.6727	8.2126	0.0286	0.1914	0.1239	0.2842
0	8.2818	8.2630	8.1767	8.8536				
0	8.3279	8.4330	8.3880	8.8767				
7	8.4007	8.4730	8.2316	7.7681	0.1408	0.1005	0.1295	0.3033
7	7.8941	8.1968	8.4114	8.2574				
7	8.0108	8.6407	7.9092	8.5376				
13	7.5193	7.7100	8.1081	7.5449	0.0295	0.0643	0.0743	0.5463
13	7.5228	7.6135	8.2529	7.9067				
13	7.3108	7.9609	8.4898	8.5751				
20	7.0458	7.2895	7.8222	7.0774	0.2136	0.2481	0.5543	0.4119
20	7.1593	7.0745	8.8449	7.3123				
20	7.6600	7.7630	8.1167	7.9541				
26	7.0738	6.7858	8.6546	7.3552	0.5223	0.0816	0.6204	0.0549
26	6.6397	7.0844	8.8678	7.4318				
26	7.6581	6.6996	7.8144	7.6728				
43	6.0966	6.6983	7.4058	7.1842	0.1393	0.0979	0.0299	0.0188
43	6.6169	6.2559	7.4418	7.0716				
43	6.4340	6.4861	7.2143	6.9912				
58	6.1876		7.1985	6.9941	0.0062		0.0147	0.0175
58	6.2987		7.2190	7.0735				
58	6.2478		7.0613	7.1804				
				SS:	1.0803	0.7838	1.5470	1.6369